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(71) Applicants *(for all designated States except US)*: THE WELLCOME TRUST LIMITED AS TRUSTEE TO THE WELLCOME TRUST [GB/GB]; 183 Euston Road, London NW1 2BE (GB). MERCK & CO., INC. [US/US]; 126 Lincoln Avenue, Rahway, NJ 07065–0907 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: TODD, John, Andrew [GB/GB]; 64 Rousham Road, Tackley, Oxon OX5 3AJ (GB). HESS, John, Wilfred [US/US]; 566 Constitution Road, Lansdale, PA 19446 (US). CASKEY, Charles, Thomas [US/US]; 6402 Bellmont, Houston, TX 66005 (US). COX, Roger, David [GB/GB]; Peace Cottages, 7 Park Road, Banbury, Oxon OX16 0DW (GB). GERHOLD, David [US/US]; 730 Tranquility Lane, Lansdale, PA 19446 (US). HAMMOND, Holly [US/US]; 621 Melvins Road, Telford, PA 18969 (US). HEY, Patricia [US/US]; 1133 Bloomfield Circle, Lansdale, PA 19446 (US). KAWAGUCHI, Yoshihiko [JP/JP]; Yamadanishi 3–33–B–206, Suita–shi, Osaka 565 (JP). MERRIMAN, Tony, Raymond [NZ/GB]; 16A Cromwell Close, Marston, Oxford OX3 0RW (GB). METZKER, Michael, Lee [US/US]; 7400 Encampment Road, Lansdale, PA 19446 (US). NAKAGAWA, Yusuke [JP/GB]; 17 Latimer Grange, Latimer Road, Headington, Oxford OX3 7PQ (GB). PHILLIPS, Michael, Sean [CA/US]; 608 Poplar Court, Lansdale, PA 19446 (US). TWELLS, Rebecca, Christina, Joan [GB/GB]; 75 Park Street, Thame, Oxon OX9 3HU (GB).

(74) Agents: WALTON, Seán, M. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).

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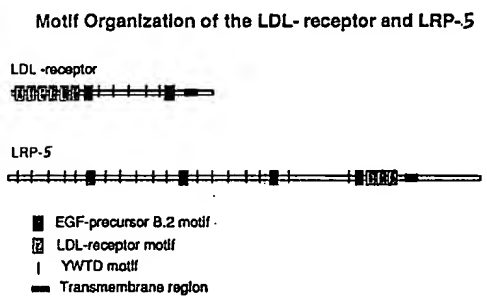

(57) Abstract

A novel receptor, "LDL–receptor related protein–3" ("LRP–3"), is provided, along with encoding nucleic acid. The gene is associated with type 1 diabetes (insulin dependent diabetes mellitus), and experimental evidence provides indication that it is the IDDM susceptibility gene *IDDM4*. In various aspects the invention provides nucleic acid, including coding sequences, oligonucleotide primers and probes, polypeptides, pharmaceutical compositions, methods of diagnosis or prognosis, and other methods relating to and based on the gene, including methods of treatment of diseases in which the gene may be implicated, including autoimmune diseases, such as glomerulonephritis, diseases and disorders involving disruption of endocytosis and/or antigen presentation, diseases and disorders involving cytokine clearance and/or inflammation, viral infection, elevation of free fatty acids or hypercholesterolemia, osteoporosis, Alzheimer's disease, and diabetes.

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NOVEL LDL-RECEPTOR

FIELD OF THE INVENTION

The present invention relates to nucleic acids, polypeptides, oligonucleotide probes and primers, methods of diagnosis or prognosis, and other methods relating to and based on the identification of a gene, which is characterised as a member of the LDL-receptor family and for which there are indications that some alleles are associated with susceptibility to insulin-dependent diabetes mellitus ("IDDM"), also known as type 1 diabetes.

More particularly, the present invention is based on cloning and characterisation of a gene which the present inventors have termed "*LDL-receptor related protein-5 (LRP5)*" (previously "*LRP-3*"), based on characteristics of the encoded polypeptide which are revealed herein for the first time and which identify it as a member of the LDL receptor family. Furthermore, experimental evidence is included herein which provides indication that *LRP5* is the IDDM susceptibility gene *IDDM4*.

BACKGROUND OF THE INVENTION

Diabetes, the dysregulation of glucose homeostasis, affects about 6% of the general population. The most serious form, type 1 diabetes, which affects up to 0.4% of European-derived population, is caused by autoimmune destruction of the insulin producing β-cells of the pancreas, with a peak age of onset of 12 years. The β-cell destruction is irreversible, and despite insulin replacement by injection patients suffer early mortality, kidney failure and blindness (Bach, 1994; Tisch and McDevitt, 1996). The major aim, therefore, of genetic research is to identify the genes predisposing to type 1 diabetes and to use this information to understand disease mechanisms and to predict and prevent the total destruction of β-cells and the disease.

The mode of inheritance of type 1 diabetes does not follow a simple Mendelian pattern, and the concordance of

susceptibility genotype and the occurrence of disease is much less than 100%, as evidenced by the 30-70% concordance of identical twins (Matsuda and Kuzuya, 1994; Kyvik et al, 1995). Diabetes is caused by a number of genes or polygenes acting together in concert, which makes it particularly difficult to identify and isolate individual genes.

The main IDDM locus is encoded by the major histocompatibility complex (MHC) on chromosome 6p21 (*IDDM1*). The degree of familial clustering at this locus, $\lambda s = 2.5$, where λs = P expected [sharing of zero alleles at the locus identical-by-descent (IBD)]/P observed [sharing of zero alleles IBD] (Risch 1987; Todd, 1994), with a second locus on chromosome 11p15, *IDDM2*, the insulin minisatellite $\lambda s = 1.25$ (Bell et al, 1984; Thomson et al, 1989; Owerbach et al, 1990; Julier et al, 1991; Bain et al, 1992; Spielman et al, 1993; Davies et al, 1994; Bennett et al, 1995). These loci were initially detected by small case control association studies, based on their status as functional candidates, which were later confirmed by further case-control, association and linkage studies.

These two loci, however, cannot account for all the observed clustering of disease in families ($\lambda s = 15$), which is estimated from the ratio of the risk for siblings of patients and the population prevalence (6%/0.4%) (Risch, 1990). We initiated a positional cloning strategy in the hope of identifying the other loci causing susceptibility to type 1 diabetes, utilising the fact that markers linked to a disease gene will show excess of alleles shared identical-by-descent in affected sibpairs (Penrose, 1953; Risch, 1990; Holmans, 1993).

The initial genome-wide scan for linkage utilising 289 microsatellite markers, in 96 UK sibpair families, revealed evidence of linkage to an additional eighteen loci (Davies et al, 1994). Confirmation of linkage to two of these loci was achieved by analysis of two additional family sets (102 UK families and 84 USA families), *IDDM4* on chromosome 11q13 (MLS 1.3 , P = 0.003 at *FGF3*) and *IDDM5* on chromosome 6q (MLS 1.8

at *ESR*). At *IDDM4* the most significant linkage was obtained in the subset of families sharing 1 or 0 alleles IBD at HLA (MLS = 2.8; P=0.001; λs = 1.2) (Davies et al, 1994). This linkage was also observed by Hashimoto *et al* (1994) using 251 affected sibpairs, obtaining P= 0.0008 in all sibpairs. Combining these results, with 596 families, provides substantial support for *IDDM4* (P = 1.5X10-6) (Todd and Farrall, 1996; Luo et al, 1996).

BRIEF DESCRIPTION OF THE INVENTION

The present inventors now disclose for the first time a gene encoding a novel member of the LDL-receptor family, which they term "*LRP5*" (previously "*LRP-3*"). Furthermore, evidence indicates that the gene represents the IDDM susceptibility locus *IDDM4*, the identification and isolation of which is a major scientific breakthrough.

Over the last 10 years many genes for single gene or monogenic diseases, which are relatively rare in the population, have been positioned by linkage analysis in families, and localised to a small enough region to allow identification of the gene. The latter sublocalisation and fine mapping can be carried out in single gene rare diseases because recombinations within families define the boundaries of the minimal interval beyond any doubt. In contrast, in common diseases such as diabetes or asthma the presence of the disease mutation does not always coincide with the development of the disease: disease susceptibility mutations in common disorders provide risk of developing of the disease, and this risk is usually much less than 100%. Hence, susceptibility genes in common diseases cannot be localised using recombination events within families, unless tens of thousands of families are available to fine map the locus. Because collections of this size are impractical, investigators are contemplating the use of association mapping, which relies on historical recombination events during the history of the population from which the families came from.

Association mapping has been used in over a dozen examples of rare single gene traits, and particularly in genetically isolated populations such as Finland to fine map disease mutations. Nevertheless, association mapping is fundamentally different from straightforward linkage mapping because even though the degree of association between two markers or a marker and a disease mutation is proportional to the physical distance along the chromosome this relationship can be unpredictable because it is dependent on the allele frequencies of the markers, the history of the population and the age and number of mutations at the disease locus. For rare, highly penetrant single gene diseases there is usually one major founder chromosome in the population under study, making it relatively feasible to locate an interval that is smaller than one that can be defined by standard recombination events within living families. The resolution of this method in monogenic diseases in which there is one main founder chromosome is certainly less than 2cM, and in certain examples the resolution is down to 100 kb of DNA (Hastbacka *et al.* (1994) Cell 78,1-20).

In common diseases like type 1 diabetes, which are caused by a number of genes or polygenes acting together in concert the population frequency of the disease allele may be very high, perhaps exceeding 50%, and there are likely to be several founder chromosomes, all of which impart risk, and not a 100% certainty of disease development. Because association mapping is dependent on unpredictable parameters, and because founder chromosomes will be several and common in frequency in the general population, the task of fine mapping polygenes is currently one of some controversy, and many doubt the feasibility at all of a systematic genetic approach using a combination of linkage and association mapping. Recently, Risch and Marakandis have provided some mathematical background to the feasibility of association mapping in complex diseases (*Science* 273 1516-1517, 1996) but they did not take into account the effect of multiple founder chromosomes.

As a result of these uncertainties, extremely large numbers of diabetic families are required for genotyping, with a large number of markers across a specific region, giving a linkage disequilibrium curve which may have several peaks. The question is, which peak identifies the aetiological mutation, and in what ways can we establish this? To our knowledge, the linkage disequilibrium curves and haplotype association maps shown in Figures 3, 4, 19 and 20 are the first of their kind for any complex polygenic disease for any locus. Curves of this nature have not been published yet in the literature, even for the well-established *IDDM1/MHC* locus. In this respect the work described here is entirely novel and at the cutting edge of research into the genetics of polygenes.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates approximate localisation of *IDDM4* on chromosome 11q13. Multipoint linkage map of maximum likelihood IBD in a subgroup of HLA 1:0 sharers in 150 families. MLS of 2.3 at *FGF3* and *D11S1883* ($\lambda s = 1.19$) were obtained (Davies *et al* (1994) *Nature* 371: 130-136).

Figure 2 shows a physical map of the region *D11S987* - *Galanin* on chromosome 11q13. The interval was cloned in pacs, bacs and cosmids, and restriction mapped using a range of restriction enzymes to determine the physical distance between each marker.

Figure 3 shows a single-point linkage disequilibrium curve at the *IDDM4* region. 1289 families were analysed by TDT, with a peak at *H0570POLYA*,) P=0.001. x-axis: physical distance in kb; y-axis: TDT $\chi 2$ statistic (tdf).

Figure 4 shows a three-point rolling linkage disequilibrium curve at *IDDM4*, with 1289 families, from four different populations (UK, USA, Sardinia and Norway). In order to minimise the effects of variation in allele frequency at each polymorphism, the TDT data was obtained at three consecutive markers, and expressed as an average of the three. x-axis: physical distance in kb; y-axis: TDT $\chi 2$ statistic.

Figure 5(a) shows DNA sequence of the *LRP5* isoform 1

cDNA.

Figure 5(b) shows the DNA sequence of the longest open reading frame present in the LRP5 cDNA.

Figure 5(c) amino acid sequence translation (in standard single letter code) of the open reading frame in Figure 5 (b).

Figure 5(d) motifs of LRP5 isoform 1, encoded by the open reading frame contained in Figure 5(b). Symbols: Underlined residues 1-24 contain a signal for protein export and cleavage, ▼ indicates the position of an intron/exon boundary, * indicates a putative N-linked glycosylation site in the proposed extracellular portion of the receptor. The EGF-binding motifs are shaded light gray, LDL-receptor ligand motifs are shaded a darker gray. The spacer regions are indicated by the underlined four amino acids with high similarity to the YWTD motif. A putative transmembrane spanning domain is underlined with a heavy line. Areas shaded in the cytoplasmic domain (1409 to end) may be involved in endocytosis.

Figure 5(e) amino acid sequence of the mature LRP5 protein.

Figure 5(f) shows the comparison of the nucleotide sequence of the first 432 nucleotides of the 5′ end of the human isoform1 cDNA sequence (Figure 5(a)) on the upper line with the first 493 nucleotides of the 5′ end of the mouse Lrp5 cDNA sequence (Figure 16(a)) on the lower line. The comparison was performed using the GCG algorithm GAP (Genetics Computer Group, Madison, WI).

Figure 5(g) shows the comparison of the first 550 amino acids of human LRP5 isoform 1 with the first 533 amino acids of mouse Lrp5 using the GCG algorithm GAP (Genetics Computer Group, Madison, WI).

Figure 6(a) shows the amino acid sequence of LRP5 motifs. A comparison was made using the program crossmatch (obtained from Dr. Phil Green, University of Washington) between the motifs present in LRP1 and the LRP5 amino acid sequence. The best match for each LRP5 motif is shown. For each motif, the top line is the LRP5 isoform 1 amino acid sequence, the

middle line is amino acids that are identical in the two motifs, the lower line is the amino acid sequence of the best match LRP1 motif. Of particular note are the conserved cysteine (C) residues that are the hallmark of both the EGF-precursor and LDL-receptor ligand binding motifs.

Figure 6(b) illustrates the motif organization of the LDL-receptor and LRP5. The LDL-receptor ligand binding motif are represented by the light gray boxes, the EGFlike motifs are represented by the dark gray boxes. The YWTD spacer motifs are indicated by the vertical lines. The putative transmembrane domains are represented by the black box.

Figure 7 shows *LRP5* gene structure. The DNA sequence of contiguous pieces of genomic DNA is represented by the heavy lines and are according to the indicated scale. The position of the markers *D11S1917(UT5620)*, *H0570POLYA*, *L3001CA*, *D11S1337*, and *D11S970* are indicated. The exons are indicated by the small black boxes with their numerical or alphabetical name below, the size of the exons is not to scale.

Figure 8 illustrates different *LRP5* gene isoforms. Alternatively spliced 5′ ends of the *LRP5* gene are indicated with the isoform number for each alternatively spliced form. The light gray arrow indicates the start of translation which occurs in exon 6 in isoform 1, may occur upstream of exon 1 in isoform 3 and occurs in exon B in isoforms 2, 4, 5. and 6. The core 22 exons (A to V) are represented by the box.

Figure 9 is a SNP map of Contig 57. Polymorphisms were identified by the comparison of the DNA sequence of BAC 14-1-15 with cosmids EO 864 and BO 7185. Corresponding Table 6 indicates a PCR amplicon that includes the site of the polymorphism, the nature of the single nucleotide polymorphis (SNP), its location and the restriction site that is altered, if any. The line represents the contiguous genomic DNA with the relative location of the polymorphisms and the amplicons used to detect them. The large thin triangles represent the site of putative exons. The marker *H0570POLYA* is indicated.

Figure 10 is a SNP map of Contig 58. Polymorphisms were identified by the comparison of the DNA sequence of BAC 14-1-

15 with cosmid BO 7185. Corresponding Table 6 indicates a PCR amplicon that includes the site of the polymorphism, the nature of the single nucleotide polymorphism (SNP), its location and the restriction site that is altered, if any. The line represents the contiguous genomic DNA with the relative location of the polymorphisms and the amplicons used to detect them. The large thin triangle at the very end of the line represents exon A of *LRP5*.

Figure 11(a) shows the DNA sequence of the isoform 2 cDNA.

Figure 11(b) shows the longest open reading frame of isoform 2 (also isoform 4,5,6).

Figure 11(c) shows the amino acid sequence of isoform 2 (also isoform 4,5,6), encoded by the open reading frame of Figure 12(b).

Figure 12(a) shows the DNA sequence of isoform 3 cDNA.

Figure 12(b) shows sequence obtained by GRAIL and a putative extension of isoform 3.

Figure 12(c) shows a putative open reading frame for isoform 3.

Figure 12(d) shows the amino acid sequence of isoform 3.

Figure 12(e) shows the GRAIL predicted promoter sequence for isoform 3.

Figure 13 shows the DNA sequence of the isoform 4 cDNA, which contains an open reading frame encoding isoform 2 (Figure 11 (b)).

Figure 14 shows the DNA sequence of the present in cDNA isoform 5, which contains an open reading frame encoding isoform 2 (Figure 11 (b)).

Figure 15 shows the DNA sequence of isoform 6, which contains an open reading frame encoding isoform 2(Figure 11 (b)).

Figure 15(b) shows the GRAIL predicted promoter sequence associated with isoform6.

Figure 16(a) shows the DNA sequence of a portion of the mouse *Lrp5* cDNA.

Figure 16(b) shows the DNA sequence of the 5' extension

of the mouse clone.

Figure 16(c) shows the DNA sequence of a portion of the open reading frame of mouse *Lrp5*.

Figure 16(d) show the amino acid sequence of the open reading frame encoding a portion of mouse Lrp5.

Figure 17(a) shows DNA sequence of exons A to V.

Figure 17(b) shows the amino acid sequence encoded by an open reading frame contained in Figure 17(a).

Figure 18 (a) shows the nucleotide sequence of the full length mouse *Lrp5* cDNA.

Figure 18 (b) shows the nucleotide sequence for the longest open reading frame present in the mouse *Lrp5* cDNA.

Figure 18 (c) shows the amino acid sequence translation (in single letter code) of the open reading frame in Figure 18 (b).

Figure 18 (d) shows an alignment of the amino acid sequence of the human LRP5 protein and the mouse Lrp5 protein program using the GCG algorithm GAP (Genetics Computer Group, Madison, WI).

Figure 18 (e) shows an alignment of the amino acid sequence of the mature human LRP5 protein with the mature mouse LRP5 program using the GCG algorithm GAP (Genetics Computer Group, Madison, WI).

Figure 19 shows a schematic representation of haplotypes across the *IDDM4* region. Three distinct haplotypes are shown. Haplotype A is protective against IDDM whereas haplotypes B and C are susceptible/non-protective for IDDM.

Figure 20 shows a schematic representation of single nucleotide polymorphism (SNP) haplotypes across the *IDDM4* region. Haplotype A is protective whereas haplotypes B, C, D, and E are susceptible/non-protective. A minimal region of 25 kb which is Identical By Descent (IBD) for the four susceptible haplotypes is indicated. The SNP designations, e.g. 57-3, are as described in Table 6 and Figures 9 and 10.

LRP5 Gene Structure

The gene identified contains 22 exons, termed A-V, which

encode most of the mature LRP5 protein. The 22 exons account for 4961 nucleotides of the *LRP5* gene transcript (Figure 5(a)) and are located in an approximately 110 kb of genomic DNA. The genomic DNA containing these exons begins downstream of the genetic marker *L3001CA* and includes the genetic markers *D11S1337*, *14lca5*, and *D11S970* (Figure 7). Several different 5' ends of the *LRP5* transcript have been identified. Of particular interest is isoform 1 with a 5' end encoding a signal peptide sequence for protein export (secretory leader peptide) across the plasma membrane. As discussed below the LRP5 protein is likely to contain a large extracellular domain, therefore it would be anticipated that this protein would have a signal sequence. The exon encoding the signal sequence, termed exon 6, lies near the genetic marker *H0570POLYA*. This exon is 35 kb upstream of exon A and thus extends the genomic DNA comprising the *LRP5* gene to at least 160kb.

Several additional isoforms of the *LRP5* gene that arise from alternative splicing of the 5' end have been identified by PCR (Figure 8). The functional relevance of these additional isoforms is not clear. Two of these *LRP5* transcripts contain exon 1 which is located upstream of the genetic marker *D11S1917(UT5620)* and expands the *LRP5* gene to approximately 180 kb of genomic DNA. The transcript termed isoform 3 consists of exon 1 spliced directly to exon A. The reading frame is open at the 5' end and thus there is the potential for additional coding information present in exons upstream of exon 1. Alternatively, centromeric extension of exon 1 to include all of the open reading frame associated with this region yields the open reading frame for isoform 3.

The second transcript that contains exon 1 also contains exon 5, which is located near the genetic marker *H0570POLYA*. The open reading frame for this isoform, isoform 2, begins in exon B and thus encodes a truncated LRP5 protein which lacks any predicted secretory leader peptide in the first 100 amino acids. There are three additional transcripts each with an open reading frame beginning in exon B and with 5' ends near

the genetic marker *L3001CA*.

Expression Profile of LRP5

Northern blot analysis indicates that the major mRNA transcript for the *LRP5* gene is approximately 5 to 5.5 kb and is most highly expressed in liver, pancreas, prostate, and placenta. Expression is also detected in skeletal muscle, kidney, spleen, thymus, ovary, lung, small intestine, and colon. Minor bands both larger and smaller than 5 kb are detected and may represent alternative splicing events or related family members.

LRP5 is a Member of the LDL-receptor Family

The gene identified in the *IDDM4* locus, *lrp5*, is a member of the LDL-receptor family. This family of proteins has several distinguishing characteristics, a large extracellular domain containing cysteine rich motifs which are involved in ligand binding, a single transmembrane spanning domain, and an "NPXY" internalization motif (Krieger and Herz (1994) Ann. Rev. Biochem. 63: 601-637). The functional role of the members of this family is the clearance of their ligands by the mechanism of receptor mediated endocytosis. This is illustrated by the most highly characterized member of the family, the LDL-receptor which is responsible for the clearance of LDL cholesterol from plasma (Goldstein, *et. al* . (1985) Ann. Rev. Cell Biol. 1: 1-39).

LRP5 is most closely related to the LDL-receptor related protein (LRP) which is also know as the alpha2-macroglobulin receptor. Translation of the open reading frame (ORF) of isoform 1 yields the LRP5 protein. Comparison of the LRP5 protein to human LRP1 using the algorithm GAP (Genetics Computer Group, Madison, WI) reveals an overall amino acid similarity of 55% and 34% identity to the region of the human LRP1 protein from amino acids 1236 to 2934. The DNA of this ORF is 45% identical to LRP1 encoding DNA as indicated by GAP. A slightly lower but significant level of similarity is seen with the megalin receptor also termed LRP2 and gp330 (Saito,

et .al.. (1994) Proc. Natl. Acad. Sci. 91: 9725-9729), as well as the *Drosophilla* vitellogenin receptor (Schonboum *et. al.* (1995) Proc. Natl. Acad. Sci. 92: 1485-1489). Similarity is also observed with other members of the LDL-receptor family including the LDL-receptor (Suedhof *et. al.* (1985) Science 228: 815-822) and the VLDL receptor (Oka *et. al.* (1994) Genomics 20: 298-300). Due to the presence of EGF-like motifs in LRP5 similarity is also observed with the EGF precursor and nidogen precursor which are not members of the LDL-receptor family.

Properties and Motifs of LRP5

The N-terminal portion of LRP5 likely has the potential for a signal sequence cleavage site. Signal sequences are frequently found in proteins that are exported across the plasma membrane (von Heijne (1994) Ann. Rev. Biophys. Biomol. Struc. 23: 167-192). In addition, other members of the LDL-receptor family contain a signal sequence for protein export.

The presence of a signal sequence cleavage site was initially identified by a comparison of the human *LRP5* with a mouse cDNA sequence that we obtained. The initial mouse partial cDNA sequence that we obtained, 1711 nucleotides (Figure 16(a)), is 87% identical over an approximately 1500 nucleotide portion to the human *LRP5* cDNA and thus is likely to be the mouse ortholog (*Lrp5*) of the human *LRP5*. The cloned portion of the mouse cDNA contains an open reading frame (Figure 16(c)) encoding 533 amino acids. The initiating codon has consensus nucleotides for efficient translation at both the -3 (purine) and +4 (G nucleotide) positions (Kozak, M. 1996, Mamalian Genome 7:563-574). A 500 amino acid of the portion of the mouse Lrp5 (Figure 5(g) and Figure 16(d)) is 96% identical to human LRP5, further supporting the proposal that this is the mouse ortholog of *LRP5*.

Significantly, the first 200 nucleotides of the mouse cDNA have very little similarity to the 5' extensions present in isoforms 2-6 discussed below. By contrast this sequence is 75% identical with the human sequence for exon 6 that

comprises the 5' end of isoform 1. Thus isoform 1 which encodes a signal peptide for protein export likely represents the most biologically relevant form of LRP5.

Importantly, both the human *LRP5* and mouse *Lrp5* open reading frames encodes a peptide with the potential to act as a eukaryotic signal sequence for protein export (von Heijne, 1994, Ann. Rev. Biophys. Biomol. Struc. 23:167-192). The highest score for the signal sequence as determined by using the SigCleave program in the GCG analysis package (Genetics Computer Group, Madison WI) generates a mature peptide beginning at residue 25 of human LRP5 and residue 29 of mouse Lrp5 (Figure 5(d and g)). Additional sites that may be utilized produce mature peptides in the human LRP5 beginning at amino acid residues 22, 23, 23, 26, 27, 28, 30 or 32. Additional cleavage sites in the mouse Lrp5 result in mature peptides beginning at amino acid residue 31, 32, 33, or 38 (Figure 5(g)). The mature human LRP5 protein is show in Figure 5(e).

The other alternative isoforms of LRP5 lack a signal sequence near the N-terminus of the encoded protein. The functional relevance of these additional isoforms is not known, however there are several exported proteins which lack a signal sequence and are transported by a signal peptide independent mechanism (Higgins, C.F. (1992) Ann. Rev. Cell Biol. 8: 67-113). Thus it is possible that the putative extracellular domain of these isoforms is translocated across the plasma membrane.

The extracellular domain of members of the LDL receptor family contains multiple motifs containing six cysteine residues within an approximately 40 amino acid region. (Krieger and Herz (1994) Ann. Rev. Biochem. 63: 601-637). Several classes of these cysteine rich motifs have been defined based on the spacing of the cysteine residues and the nature of other conserved amino acids within the motif. The LDL-receptor ligand binding (class A) motif is distinguished by a cluster of acidic residues in the C-terminal portion of the motif which includes a highly conserved SDE sequence. The

importance of this acidic region in ligand binding has been demonstrated by mutagenesis studies (Russell *et. al.* (1989) J. Biol. Chem. 264: 21682-21688). Three LDL-receptor ligand binding motifs are found in the LRP5 protein (Figure 6(a)). The EGF-like (class B) motif lacks the cluster of acidic residues present in the LDL-receptor ligand binding motif. In addition, the spacing of the cysteine residues differs in the EGF-like motifs relative to the LDL-receptor ligand binding motif. The LRP5 protein contains 4 EGF-precursor (B.2) motifs,which have the property of an NGGCS motif between the first and second cysteine residue (Figure 6(a)).

The size of the members of the LDL receptor family and the number of the cysteine-rich repeats in the extracellular domain varies greatly. LRP1 is a large protein of 4544 amino acids and contains 31 LDL-receptor ligand binding motifs (class A) and 22 EGF-like motifs (class B) (Herz *et. al.*, (1988) EMBO 7: 4119-4127). Similarly the megalin receptor, LRP2, is a protein of 4660 amino acids and consists of 36 LDL-receptor ligand binding motifs and 17 EGF-like motifs (Saito *et. al.* (1994) PNAS 91: 9725-9729). In contrast, the LDL receptor is a relatively small protein of 879 amino acids which contains 7 LDL-ligand binding motifs and 3 EGF-like motifs. The predicted size of the mature LRP5 protein, 1591 amino acids, is intermediate between LRP1 and the LDL receptor. As indicated above the LRP5 protein contains four EGF-like motifs and three LDL-ligand binding motifs. It has been postulated that the multiple motif units, particularly evident in LRP1 and LRP2, account for the ability of these proteins to bind multiple lipoprotein and protein ligands (Krieger and Herz (1994) Ann. Rev. Biochem. 63: 601-637).

The arrangement of the LDL-receptor ligand binding and EGF-like motifs relative to each other is similar in both the LDL receptor, LRP1, and LRP2. In each of these proteins multiple LDL-ligand binding motifs are grouped together and followed by at least one EGF-like motif (Herz *et. al.*, (1988) EMBO 7: 4119-4127, 1988). By contrast, in the LRP5 protein an EGF-like motif precedes the group of three LDL-ligand binding

motifs (Figure 6(b)). An additional property unique to LRP5 is that the LDL-ligand binding motifs in LRP5 are followed by the putative transmembrane domain. The different arrangement of the motifs may define LRP5 as a member of a new subfamily within the LDL-receptor related protein family.

LRP5 has a signal peptide for protein export at the N-terminus of the protein. Signal peptide cleavage yields a mature LRP5 protein which begins with an EGF precursor spacer domain from amino acids 31-297 (amino acid residue numbers are based upon the LRP5 precursor). The EGF precursor spacer domain is composed of five approximately 50 amino acid repeats that each contain the characteristic sequence motif Tyr-Trp-Thr-Asp (YWTD). There are three additional spacer domains from amino acids 339-602, 643-903, and 944-1214. Each spacer domain is followed by an EGF repeat from amino acids 297-338 (egf1), 603-642 (egf2), 904-943 (egf3), and 1215-1255 (egf4). The EGF repeats contain six conserved cysteine residues and are of the B.2 class which has an Asn-Gly-Gly-Cys (NGGC) motif as a feature (Herz *et al.* 1988, EMBO J 7:4119-27) (Figure 6(a)). A single unit defined as an EGF precursor spacer domain and an EGF repeat, is repeated four times in LRP5. The last EGF repeat is adjacent to three consecutive LDLR repeats from amino acids 1257-1295(ldlr1), 1296-1333 (ldlr2), and 1334-1372 (ldlr3). The LDLR repeats have the conserved cysteine residues, as well as, the motif Ser-Asp-Glu (SDE) as a characteristic feature (Figure 6(a)). There are thirteen amino acids separating the LDLR repeats from the putative transmembrane spanning domain of 23 amino acids from 1386-1408. The putative extracellular domain of LRP5 has six potential sites for N-linked glycosylation at amino acid residues 93, 138, 446, 499, 705, and 878 (Figure 5(d)).

The intracellular domain of LRP5 is comprised of 207 amino acids which is longer than most members of the family but similar in size to LRP2 (Saito *et. al.* (1994) PNAS 91:9725-9729). It does not exhibit similarity to the LDL-

receptor family, nor is it similar to any other known proteins. The cytoplasmic domain of LRP5 is comprised of 16% proline and 15% serine residues (Figure 5(d)). Most members of the LDL-receptor family contain a conserved NPXY motif in the cytoplasmic domain which has been implicated in endocytosis by coated pits (Chen *et. al.* (1990) J. Biol. Chem. 265: 3116-3123). Mutagenesis studies have indicated that the critical residue for recognition by components of the endocytotic process is the tyrosine residue (Davis, *et al.* (1987) Cell 45: 15-24). Replacement of the tyrosine residue by phenylalanine or tryptophan is tolerated, thus the minimal requirement for this residue appears to be that it is aromatic amino acid (Davis, *et al.* (1987) Cell 45: 15-24). Structural studies have indicated that the critical function of the NP residues is to provide a beta-turn that presents the aromatic residue (Bansal and Gierasch (1991) Cell 67: 1195-1201).

Although the cytoplasmic domain of LRP5 does not contain an NPXY motif, there are several aromatic residues in the LRP5 cytoplasmic domain that lie in putative turn regions (Figure 5(d)) and thus may be involved in facilitating endocytosis. In particular tyrosine 1473 which occurs in the sequence VPLY motif has the proline and tyrosine in the correct position, relative to the consensus motif. Although the NPXY motif has been implicated in endocytosis in several proteins it is not an absolute requirement as there are proteins that lack the NPXY motif, e.g. the transferrin receptor, that undergo endocytosis by coated pits (Chen, *et. al.* (1990) J. Biol. Chem. 265: 3116-3123). In any event, we anticipate that the primary function of this protein will be receptor mediated endocytosis of its ligand.

Potential Roles of LRP5

The ability of members of the LDL-receptor family to bind multiple ligands suggests that LRP5 may function to bind one or more ligands. Moreover, in a fashion analogous to other members of the family, once bound the LRP5 receptor ligand complex would endocytose resulting in clearance of the ligand

from the extracellular milieu. The nature of the LRP5 ligand may be a lipid, a protein, a protein complex, or a lipoprotein and may possess a variety of functions. Although the physiological function of the most closely related member of the LDL-receptor family, LRP1, is uncertain, it does possess a number of biochemical activities. LRP1 binds to alpha-2 macroglobulin. Alpha-2 macroglobulin is a plasma complex that contains a "bait" ligand for a variety of proteinases e.g. trypsin, chymotrypsin, pancreatic elastase and plasma kallikrein (Jensen (1989) J. Biol. Chem. 20:11539-11542). Once the proteinase binds and enzymatically cleaves the "bait" alpha-2 macroglobulin undergoes a conformational change and "traps" the proteinase. The proteinase:alpha-2 macroglobulin complex is rapidly cleared by LRP. This mechanism scavenges proteinases that have the potential to mediate a variety of biological functions e.g. antigen processing and proteinase secretion (Strickland *et. al.* (1990) J. Biol. Chem. 265: 17401-17404). The importance of this function is evidenced by the prenatal death of *Lrp1* knockout mice (Zee *et. al.* (1994) Genomics 23: 256-259).

Antigen presentation is a critical component in the development of IDDM as is evidenced by the pivotal role of MHC haplotypes in conferring disease susceptibility (Tisch and McDivitt (1996) Cell 85: 291-297). By analogy with LRP1, LRP5 may play a role in antigen presentation in which case polymorphisms within this gene could affect the development of autoimmunity in the type 1 diabetic patient.

The alpha-2 macroglobulin complex also binds cytokines and growth factors such as interleukin-1 beta, interleukin 2, interleukin 6, transforming growth factor-beta, and fibroblast growth factor (Moestrup and Gliemann (1991) J. Biol. Chem. 266: 14011-14017). Thus the alpha-2 macroglobulin receptor has the potential to play a role in the clearance of cytokines and growth factors. The role of cytokines in mediating immune and inflammatory responses is well established. For example, the interleukin-2 gene is a strong candidate gene for the *Idd3* locus in the non-obese diabetic mouse, an animal model for

type 1 diabetes (Denny *et. al.* (1977) Diabetes 46:695-700). If LRP5 binds alpha-2 macroglobulin or related complexes then it may play a role in the immune response by mediating cytokine clearance. For example, the LRP5 which is expressed in pancreas, the target tissue of IDDM, may play a role in clearing cytokines from the inflammatory infiltrate (insulitis) that is ongoing in the disease. A polymorphism in LRP5 that reduces the ability of LRP5 to clear cytokines may increase an individuals susceptibility to developing IDDM. Furthermore an individual with a polymorphism that increases the ability of LRP5 to clear cytokines may be protected from developing IDDM. Conversely, certain cytokines counteract other cytokines and thus removal of certain beneficial cytokines by LRP5 may confer disease susceptibility and thus a polymorphism that reduces LRP5 activity may confer protection from developing the disease.

Increases of free fatty acids (FFA) have been shown to reduce insulin secretion in animals (Boden *et. al.* (1997) Diabetes 46: 3-10). In addition, ApoE which is a ligand for the LDL-receptor, has been associated with an antioxidant activity (Miyata and Smith (1996) Nature Genet. 14: 55-61) and oxidative damage is a central pathogenic mechanism in pancreatic β-cell destruction in type 1 diabetes (Bac (1994) Endocrin. Rev. 15: 516-542). Thus alterations in the ability of LRP5 to bind ApoE and related lipoproteins may influence the susceptibility to oxidative damage in pancreatic β-cells. Transfection of forms of LRP5 into β-cells may facilitate resistance of β cells to damage by the immune system in autoimmunity and in transplantation.

A pharmacological entity termed the lipolysis-stimulated receptor (LSR) which binds and endocytoses chylomicron remnants in the presence of FFA has been described (Mann *et. al.*. (1995) Biochemistry 34: 10421-10431. One possible role for the *LRP5* gene product is that it is responsible for this activity.

Another member of the LRP family is LRP2, also known as megalin and gp330, this protein has been implicated in

Heymann's nephritis, an autoimmune disease of the kidney in rats (Saito *et. al.* (1994) PNAS 91: 9725-9729). Heymann's nephritis is a model of glomerularnephritis and is characterized by the development of autoantibodies to the alpha-2 macroglobulin receptor associated protein, also known as the Heymann nephritis antigen. The Heymann nephritis antigen binds to LRP2 (Strickland *et. al.* (1991) J. Biol. Chem. 266: 13364-13369). LRP2 may play a role in this disease by clearance of this pathogenic protein. In an analogous manner the function of LRP5 may be to bind and clear proteins in the pancreas to which the IDDM patient has generated autoantibodies. Alternatively LRP5 itself may be an autoantigen in the IDDM patient.

LRP1 has been identified as the receptor for certain bacterial toxins (Krieger and Herz (1994) Ann. Rev. Biochem. 63: 601-637) and the human rhinovirus (Hofer *et. al.* (1994) Proc. Natl. Acad. Sci. 91: 1839-42). It is possible that a viral infection alters an individuals susceptibility to IDDM (Epstein (1994) N. Eng. J. Med. 331: 1428-1436). If certain viruses utilize LRP5 as a mode of entry into the cell then polymorphisms in LRP5 may alter the individuals susceptibility to type 1 diabetes.

Alterations in LRP5 may participate in the pathogenesis of other diseases. LRP1 binds lipoproteins such as apoE and C-apolipoproteins. The clearance of lipoproteins such as apoE and apoB by the LDL receptor is its primary role, mutations in the LDL receptor lead to hypercholesterolemia (Chen *et. al.* (1990) J. Biol. Chem. 265: 3116-3123). Therefore mutations in LRP5 that decrease the ability of the protein to scavenge lipoproteins may cause an elevation in cholesterol. Variations in LRP5 could predispose to the development of macrovascular complications in diabetics, the major cause of death. In type 2 diabetics, pancreatic pathology is characterised by the deposition of amyloid. Amyloid deposition may decrease pancreatic β-cell function. LRP5 could function in the metabolism of islet amyloid and influence susceptibility to type 2 diabetes as well as type 1

diabetes. The role of ApoE in Alzheimer's disease indicates that proteins such as LRP1 and possibly LRP5 have the potential to contribute to the pathogenesis of this disease.

Polymorphism in genes involved in the development of osteoporosis-pseudoglioma syndrome have been mapped to a 3-cM region of chromosome 11 which includes the gene encoding LRP5 (Gong *et. al.* (1996) Am. J. Hum. Genet. 59: 146-151). The pathogenic mechanism of this disease is unknown but is believed to involve a regulatory role, patients with have aberrant vascular growth in the vitero-retina. The potential role of LRP5 in the clearance of fibroblast growth factor, a mediator of angiogenesis, and the chromosomal location of the gene suggests that it may play a role in this disease. This proposed function could also be connected with the development of retinopathy in diabetes.

Polymorphisms in the LRP5 Gene

The exons of the *LRP5* gene are being scanned for polymorphisms. There are several polymorphisms that change an amino acid in LRP5 that have been identified in IDDM patients (Table 5). Of particular interest is a C to T transition, which changes an Ala codon to Val, in one of the three conserved LDL receptor ligand binding motifs. In addition to this polymorphism described above, a C to T transition was identified in the codon for Asn^{709} (with no effect on the encoded amino acid), and three polymorphisms were identified in intronic sequences flanking the exons. An additional set of polymorphisms has been identified by comparing experimentally derived cDNA sequences with the genomic DNA sequence (Table 5). Some of these polymorphism will be analyzed in a large number of IDDM patients and control individuals to determine their association with IDDM.

A number of (approximately 30) single nucleotide polymorphisms (SNPs) were identified in the genomic DNA sequences of overlapping BAC and cosmid clones surrounding the genetic marker poly A. The contiguous genomic sequences containing these polymorphism have been termed contig 57 (Figure 9), which contains exons 1 and 5 along with the

genetic markers poly A and *D11S1917(UT5620)*, and contig 58 (Figure 10) which contains the genetic marker *L3001ca* and part of exon A.

Additional Experimental Evidence

A region of identity-by-descent associated with type 1 diabetes has been identified in the 5' portion of the *LRP5* gene. By combining data from SNPs and microsatellite markers we have identified a region identical-by-descent in susceptible haplotypes, the minimal region consists of 25 kb which contains the putative regulatory regions of *LRP5* and the first exon. This strengthens the genetic evidence for *LRP5* being a diabetes risk gene. Therefore therapies that affect *LRP5* may be useful in the prevention and treatment of type 1 diabetes.

Overexpression of *LRP5* in mice provides evidence for LRP5 affecting lipoprotein metabolism. Statistically significant evidence for modulation of triglycerides by LRP5 has been obtained. Thus therapies that affect *LRP5* may be useful in the treatment of cardiovascular disease and conditions where serum triglycerides are elevated.

Suggestive evidence was obtained for LRP5 reducing serum cholesterol when it is above normal. There is also evidence for the ability of LRP5 to interact with very low-density lipoprotein particles and reduce their levels in serum. Therefore therapies that affect *LRP-5* may be useful in the treatment of cardiovascular disease and conditions where serum cholesterol levels are elevated.

Biochemical studies indicate that LRP5 has the capacity to function in the uptake of low-density lipoprotein (LDL) particles. Thus therapies that affect *LRP5* may be useful in the treatment of cardiovascular disease where LDL levels are elevated.

Overexpression of *LRP5* in mice provided statistically significant evidence for a reduction in serum alkaline phosphatase. A reduction in serum alkaline phosphatase is consistent with LRP5 playing a role in modulation of the immune response. This provides evidence for *LRP5* participating in the pathogenesis of type 1 diabetes.

Therefore therapies that affect LRP5 may be useful in the treatment of autoimmune diseases.

Cellular localization of LRP5 indicates that it is expressed in a particular subtype, the phagocytic macrophages, of mature tissue macrophages. Evidence from the literature indicates that this class of macrophages is involved in autoimmune disease, supporting a role for LRP5 in autoimmune disease and type 1 diabetes. Therefore therapies that affect *LRP5* may be useful in the treatment of autoimmune diseases.

Full length cDNAs for both human and mouse *LRP5* have been obtained. Antibodies directed against LRP5 have been developed. These reagents provide tools to further analyze the biological function of LRP5.

Irrespective of LRP5's actual mode of action and involvement in IDDM and other diseases, the experimental work described herein establishes and supports the practical applications which are disclosed as aspects and embodiments of the present invention.

According to one aspect of the present invention there is provided a nucleic acid molecule which has a nucleotide sequence encoding a polypeptide which includes the amino acid sequence shown in Figure 5(c), Figure 5(d) or Figure 5(e). The amino acid sequence of Figure 5(c) includes that of Figure 5(e) and a signal sequence.

The coding sequence may be that shown included in Figure 5(a) or Figure 5(b) or it may be a mutant, variant, derivative or allele of the sequence shown. The sequence may differ from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to a nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

Thus, nucleic acid according to the present invention may include a sequence different from the sequence shown in Figure 5(a) or Figure 5(b) yet encode a polypeptide with the same amino acid sequence. The amino acid sequence shown in Figure 5(c) consists of 1615 residues.

On the other hand the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in Figure 5(c). Nucleic acid encoding a polypeptide which is an amino acid sequence mutant, variant, derivative or allele of the sequence shown in Figure 5(c) is further provided by the present invention. Such polypeptides are discussed below. Nucleic acid encoding such a polypeptide may show at the nucleotide sequence and/or encoded amino acid level greater than about 60% homology with the coding sequence shown in Figure 5(a) and/or the amino acid sequence shown in Figure 5(c), greater than about 70% homology, greater than about 80% homology, greater than about 90% homology or greater than about 95% homology. For amino acid "homology", this may be understood to be similarity (according to the established principles of amino acid similarity, e.g. as determined using the algorithm GAP (Genetics Computer Group, Madison, WI) or identity. GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, the default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of either of the terms "homology" and "homologous" herein does not imply any necessary evolutionary relationship between compared sequences, in keeping for example with standard use of terms such as "homologous recombination" which merely requires that two nucleotide sequences are sufficiently similar to recombine under the appropriate conditions. Further discussion of polypeptides according to the present invention, which may be encoded by nucleic acid according to the present invention, is found below.

The present invention extends to nucleic acid that hybridizes with any one or more of the specific sequences disclosed herein under stringent conditions. Suitable conditions include, e.g. for detection of sequences that are about 80-90% identical such as detection of mouse *LRP5* with a human probe or vice versa, hybridization overnight at 42°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of

sequences that are greater than about 90% identical, suitable conditions include hybridization overnight at 65°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

The coding sequence may be included within a nucleic acid molecule which has the sequence shown in Figure 5(a) (isoform 1) or Figure 5(b) and encode the full polypeptide of isoform 1 (Figure 5(c)). Mutants, variants, derivatives and alleles of these sequences are included within the scope of the present invention in terms analogous to those set out in the preceding paragraph and in the following disclosure.

Also provided by the present invention in various aspects and embodiments is a nucleic acid molecule encoding a polypeptide which includes the amino acid sequence shown in Figure 17(b)This sequence forms a substantial part of the amino acid sequence shown in Figure 5(e). Nucleic acid encoding a polypeptide which includes the amino acid sequence shown in Figure 17(b) may include the coding sequence shown in Figure 17(b), or an allele, variant, mutant or derivative in similar terms to those discussed above and below for other aspects and embodiments of the present invention.

According to various aspects of the present invention there are also provided various isoforms of the LRP5 polypeptide and gene. The gene of Figure 5 is known as isoform 1. Included within the present invention is a nucleic acid molecule which has a nucleotide sequence encoding a polypeptide which includes the amino acid sequence of a polypeptide shown in Figure 11(c) (isoform 2). The coding sequence may be as shown in Figure 11(b) (which may be included within a molecule which has the sequence shown in Figure 11(a) (isoform 2) or the sequence shown in Figure 12(a) (isoform 3)), Figure 13 (isoform 4), Figure 14 (isoform 5) and Figure 15 (isoform 6). Mutants, derivatives, variants and alleles of these sequences are also provided by the present invention, as disclosed.

Further nucleic acid molecules according to the present invention include the nucleotide sequence of any of Figure 5(a), Figure 12(b), Figure 12(e), Figure 15(b), Figure 16(a) and Figure 16(b) and nucleic acid encoding the amino acid

sequences encoded by Figure 5(a), Figure 11(b), Figure 12(c) or Figure 16(c), along with mutants, alleles, variants and derivatives of these sequences. Further included are nucleic acid molecules encoding the amino acid sequence of Figure 18(c), particularly including the coding sequence shown in Figure 18(b).

Particular alleles according to the present invention have sequences have a variation indicated in Table 5 or Table 6. One or more of these may be associated with susceptibility to IDDM or other disease. Alterations in a sequence according to the present invention which are associated with IDDM or other disease may be preferred in accordance with embodiments of the present invention. Implications for screening, e.g. for diagnostic or prognostic purposes, are discussed below.

Generally, nucleic acid according to the present invention is provided as an isolate, in isolated and/or purified form, or free or substantially free of material with which it is naturally associated, such as free or substantially free of nucleic acid flanking the gene in the human genome, except possibly one or more regulatory sequence(s) for expression. Nucleic acid may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA. The coding sequence shown herein is a DNA sequence. Where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as encompassing reference to the RNA equivalent, with U substituted for T.

Nucleic acid may be provided as part of a replicable vector, and also provided by the present invention are a vector including nucleic acid as set out above, particularly any expression vector from which the encoded polypeptide can be expressed under appropriate conditions, and a host cell containing any such vector or nucleic acid. An expression vector in this context is a nucleic acid molecule including nucleic acid encoding a polypeptide of interest and appropriate regulatory sequences for expression of the polypeptide, in an *in vitro* expression system, e.g. reticulocyte lysate, or *in vivo*, e.g. in eukaryotic cells such

as COS or CHO cells or in prokaryotic cells such as *E. coli*. This is discussed further below.

The nucleic acid sequence provided in accordance with the present invention is useful for identifying nucleic acid of interest (and which may be according to the present invention) in a test sample. The present invention provides a method of obtaining nucleic acid of interest, the method including hybridisation of a probe having the sequence shown in any of Figures 5(a), 11(a), 11(b), 12(a), 12(b), 12(c), 12(e), 13, 14, 15, 15(b) 16(a), 16(b), and 16(c), or a complementary sequence, to target nucleic acid. Hybridisation is generally followed by identification of successful hybridisation and isolation of nucleic acid which has hybridised to the probe, which may involve one or more steps of PCR. It will not usually be necessary to use a probe with the complete sequence shown in any of these figures. Shorter fragments, particularly fragments with a sequence encoding the conserved motifs (Figure 5(c,d), and Figure 6(a)) may be used.

Nucleic acid according to the present invention is obtainable using one or more oligonucleotide probes or primers designed to hybridise with one or more fragments of the nucleic acid sequence shown in any of the figures, particularly fragments of relatively rare sequence, based on codon usage or statistical analysis. A primer designed to hybridise with a fragment of the nucleic acid sequence shown in any of the figures may be used in conjunction with one or more oligonucleotides designed to hybridise to a sequence in a cloning vector within which target nucleic acid has been cloned, or in so-called "RACE" (rapid amplification of cDNA ends) in which cDNA's in a library are ligated to an oligonucleotide linker and PCR is performed using a primer which hybridises with a sequence shown and a primer which hybridises to the oligonucleotide linker.

Such oligonucleotide probes or primers, as well as the full-length sequence (and mutants, alleles, variants and derivatives) are also useful in screening a test sample containing nucleic acid for the presence of alleles, mutants and variants, with diagnostic and/or prognostic implications

as discussed in more detail below.

Nucleic acid isolated and/or purified from one or more cells (e.g. human) or a nucleic acid library derived from nucleic acid isolated and/or purified from cells (e.g. a cDNA library derived from mRNA isolated from the cells), may be probed under conditions for selective hybridisation and/or subjected to a specific nucleic acid amplification reaction such as the polymerase chain reaction (PCR) (reviewed for instance in "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, 1990, Academic Press, New York, Mullis et al, Cold Spring Harbor Symp. Quant. Biol., 51:263, (1987), Ehrlich (ed), PCR technology, Stockton Press, NY, 1989, and Ehrlich et al, Science, 252:1643-1650, (1991)). PCR comprises steps of denaturation of template nucleic acid (if double-stranded), annealing of primer to target, and polymerisation. The nucleic acid probed or used as template in the amplification reaction may be genomic DNA, cDNA or RNA. Other specific nucleic acid amplification techniques include strand displacement activation, the QB replicase system, the repair chain reaction, the ligase chain reaction and ligation activated transcription. For convenience, and because it is generally preferred, the term PCR is used herein in contexts where other nucleic acid amplification techniques may be applied by those skilled in the art. Unless the context requires otherwise, reference to PCR should be taken to cover use of any suitable nucleic amplification reaction available in the art.

In the context of cloning, it may be necessary for one or more gene fragments to be ligated to generate a full-length coding sequence. Also, where a full-length encoding nucleic acid molecule has not been obtained, a smaller molecule representing part of the full molecule, may be used to obtain full-length clones. Inserts may be prepared from partial cDNA clones and used to screen cDNA libraries. The full-length clones isolated may be subcloned into expression vectors and activity assayed by transfection into suitable host cells, e.g. with a reporter plasmid.

A method may include hybridisation of one or more (e.g. two) probes or primers to target nucleic acid. Where the

nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation. A screening procedure, chosen from the many available to those skilled in the art, is used to identify successful hybridisation events and isolated hybridised nucleic acid.

Binding of a probe to target nucleic acid (e.g. DNA) may be measured using any of a variety of techniques at the disposal of those skilled in the art. For instance, probes may be radioactively, fluorescently or enzymatically labelled. Other methods not employing labelling of probe include examination of restriction fragment length polymorphisms, amplification using PCR, RN'ase cleavage and allele specific oligonucleotide probing. Probing may employ the standard Southern blotting technique. For instance DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probe may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells.

Preliminary experiments may be performed by hybridising under low stringency conditions various probes to Southern blots of DNA digested with restriction enzymes. Suitable conditions would be achieved when a large number of hybridising fragments were obtained while the background hybridisation was low. Using these conditions nucleic acid libraries, e.g. cDNA libraries representative of expressed sequences, may be searched. Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on. On the basis of amino acid sequence information, oligonucleotide probes or primers may be designed, taking into account the degeneracy of the genetic code, and, where

appropriate, codon usage of the organism from the candidate nucleic acid is derived. An oligonucleotide for use in nucleic acid amplification may have about 10 or fewer codons (e.g. 6, 7 or 8), i.e. be about 30 or fewer nucleotides in length (e.g. 18, 21 or 24). Generally specific primers are upwards of 14 nucleotides in length, but need not be than 18-20. Those skilled in the art are well versed in the design of primers for use processes such as PCR. Various techniques for synthesizing oligonucleotide primers are well known in the art, including phosphotriester and phosphodiester synthesis methods.

Preferred amino acid sequences suitable for use in the design of probes or PCR primers may include sequences conserved (completely, substantially or partly) encoding the motifs present in LRP5 (Figure 5(d).

A further aspect of the present invention provides an oligonucleotide or polynucleotide fragment of the nucleotide sequence shown in any of the figures herein providing nucleic acid according to the present invention, or a complementary sequence, in particular for use in a method of obtaining and/or screening nucleic acid. Some preferred oligonucleotides have a sequence shown in Table 2, Table 4, Table 7, Table 8 or Table 9, or a sequence which differs from any of the sequences shown by addition, substitution, insertion or deletion of one or more nucleotides, but preferably without abolition of ability to hybridise selectively with nucleic acid in accordance with the present invention, that is wherein the degree of similarity of the oligonucleotide or polynucleotide with one of the sequences given is sufficiently high.

In some preferred embodiments, oligonucleotides according to the present invention that are fragments of any of the sequences shown, or any allele associated with IDDM or other disease susceptibility, are at least about 10 nucleotides in length, more preferably at least about 15 nucleotides in length, more preferably at least about 20 nucleotides in length. Such fragments themselves individually represent aspects of the present invention. Fragments and other oligonucleotides may be used as primers or probes as discussed

but may also be generated (e.g. by PCR) in methods concerned with determining the presence in a test sample of a sequence indicative of IDDM or other disease susceptibility.

Methods involving use of nucleic acid in diagnostic and/or prognostic contexts, for instance in determining susceptibility to IDDM or other disease, and other methods concerned with determining the presence of sequences indicative of IDDM or other disease susceptibility are discussed below.

Further embodiments of oligonucleotides according to the present invention are anti-sense oligonucleotide sequences based on the nucleic acid sequences described herein. Anti-sense oligonucleotides may be designed to hybridise to the complementary sequence of nucleic acid, pre-mRNA or mature mRNA, interfering with the production of polypeptide encoded by a given DNA sequence (e.g. either native polypeptide or a mutant form thereof), so that its expression is reduce or prevented altogether. Anti-sense techniques may be used to target a coding sequence, a control sequence of a gene, e.g. in the 5' flanking sequence, whereby the antisense oligonucleotides can interfere with control sequences. Anti-sense oligonucleotides may be DNA or RNA and may be of around 14-23 nucleotides, particularly around 15-18 nucleotides, in length. The construction of antisense sequences and their use is described in Peyman and Ulman, Chemical Reviews, 90:543-584, (1990), and Crooke, Ann. Rev. Pharmacol. Toxicol., 32:329-376, (1992).

Nucleic acid according to the present invention may be used in methods of gene therapy, for instance in treatment of individuals with the aim of preventing or curing (wholly or partially) IDDM or other disease. This may ease one or more symptoms of the disease. This is discussed below.

Nucleic acid according to the present invention, such as a full-length coding sequence or oligonucleotide probe or primer, may be provided as part of a kit, e.g. in a suitable container such as a vial in which the contents are protected from the external environment. The kit may include instructions for use of the nucleic acid, e.g. in PCR and/or a

method for determining the presence of nucleic acid of interest in a test sample. A kit wherein the nucleic acid is intended for use in PCR may include one or more other reagents required for the reaction, such as polymerase, nucleosides, buffer solution etc. The nucleic acid may be labelled. A kit for use in determining the presence or absence of nucleic acid of interest may include one or more articles and/or reagents for performance of the method, such as means for providing the test sample itself, e.g. a swab for removing cells from the buccal cavity or a syringe for removing a blood sample (such components generally being sterile).

According to a further aspect, the present invention provides a nucleic acid molecule including a *LRP5* gene promoter.

In another aspect, the present invention provides a nucleic acid molecule including a promoter, the promoter including the sequence of nucleotides shown in Figure 12(e) or Figure 15(b). The promoter may comprise one or more fragments of the sequence shown in Figure 12(e) or Figure 15(b), sufficient to promote gene expression. The promoter may comprise or consist essentially of a sequence of nucleotides 5' to the *LRP5* gene in the human chromosome, or an equivalent sequence in another species, such as the mouse.

Any of the sequences disclosed in the figures herein may be used to construct a probe for use in identification and isolation of a promoter from a genomic library containing a genomic *LRP5* gene. Techniques and conditions for such probing are well known in the art and are discussed elsewhere herein. To find minimal elements or motifs responsible for tissue and/or developmental regulation, restriction enzyme or nucleases may be used to digest a nucleic acid molecule, followed by an appropriate assay (for example using a reporter gene such as luciferase) to determine the sequence required. A preferred embodiment of the present invention provides a nucleic acid isolate with the minimal nucleotide sequence shown in Figure 12(e) or Figure 15(b) required for promoter activity.

As noted, the promoter may comprise one or more sequence motifs or elements conferring developmental and/or tissue-

specific regulatory control of expression. Other regulatory sequences may be included, for instance as identified by mutation or digest assay in an appropriate expression system or by sequence comparison with available information, e.g. using a computer to search on-line databases.

By "promoter" is meant a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of double-stranded DNA).

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional initiation regulation" of the promoter.

The present invention extends to a promoter which has a nucleotide sequence which is allele, mutant, variant or derivative, by way of nucleotide addition, insertion, substitution or deletion of a promoter sequence as provided herein. Preferred levels of sequence homology with a provided sequence may be analogous to those set out above for encoding nucleic acid and polypeptides according to the present invention. Systematic or random mutagenesis of nucleic acid to make an alteration to the nucleotide sequence may be performed using any technique known to those skilled in the art. One or more alterations to a promoter sequence according to the present invention may increase or decrease promoter activity, or increase or decrease the magnitude of the effect of a substance able to modulate the promoter activity.

"Promoter activity" is used to refer to ability to initiate transcription. The level of promoter activity is quantifiable for instance by assessment of the amount of mRNA produced by transcription from the promoter or by assessment of the amount of protein product produced by translation of mRNA produced by transcription from the promoter. The amount of a specific mRNA present in an expression system may be determined for example using specific oligonucleotides which are able to hybridise with the mRNA and which are labelled or may be used in a specific amplification reaction such as the polymerase chain reaction. Use of a reporter gene facilitates

determination of promoter activity by reference to protein production.

Further provided by the present invention is a nucleic acid construct comprising a *LRP5* promoter region or a fragment, mutant, allele, derivative or variant thereof able to promoter transcription, operably linked to a heterologous gene, e.g. a coding sequence. A "heterologous" or "exogenous" gene is generally not a modified form of *LRP5*. Generally, the gene may be transcribed into mRNA which may be translated into a peptide or polypeptide product which may be detected and preferably quantitated following expression. A gene whose encoded product may be assayed following expression is termed a "reporter gene", i.e. a gene which "reports" on promoter activity.

The reporter gene preferably encodes an enzyme which catalyses a reaction which produces a detectable signal, preferably a visually detectable signal, such as a coloured product. Many examples are known, including β-galactosidase and luciferase. β- galactosidase activity may be assayed by production of blue colour on substrate, the assay being by eye or by use of a spectro-photometer to measure absorbance. Fluorescence, for example that produced as a result of luciferase activity, may be quantitated using a spectrophotometer. Radioactive assays may be used, for instance using chloramphenicol acetyltransferase, which may also be used in non-radioactive assays. The presence and/or amount of gene product resulting from expression from the reporter gene may be determined using a molecule able to bind the product, such as an antibody or fragment thereof. The binding molecule may be labelled directly or indirectly using any standard technique.

Those skilled in the art are well aware of a multitude of possible reporter genes and assay techniques which may be used to determine gene activity. Any suitable reporter/assay may be used and it should be appreciated that no particular choice is essential to or a limitation of the present invention.

Nucleic acid constructs comprising a promoter (as disclosed herein) and a heterologous gene (reporter) may be

employed in screening for a substance able to modulate activity of the promoter. For therapeutic purposes, e.g. for treatment of IDDM or other disease, a substance able to up-regulate expression of the promoter may be sought. A method of screening for ability of a substance to modulate activity of a promoter may comprise contacting an expression system, such as a host cell, containing a nucleic acid construct as herein disclosed with a test or candidate substance and determining expression of the heterologous gene.

The level of expression in the presence of the test substance may be compared with the level of expression in the absence of the test substance. A difference in expression in the presence of the test substance indicates ability of the substance to modulate gene expression. An increase in expression of the heterologous gene compared with expression of another gene not linked to a promoter as disclosed herein indicates specificity of the substance for modulation of the promoter.

A promoter construct may be introduced into a cell line using any technique previously described to produce a stable cell line containing the reporter construct integrated into the genome. The cells may be grown and incubated with test compounds for varying times. The cells may be grown in 96 well plates to facilitate the analysis of large numbers of compounds. The cells may then be washed and the reporter gene expression analysed. For some reporters, such as luciferase the cells will be lysed then analysed.

Following identification of a substance which modulates or affects promoter activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

Thus, the present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of promoter activity, in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising

such a substance, a method comprising administration of such a composition to a patient, e.g. for increasing *LRP5* expression for instance in treatment (which may include preventative treatment) of IDDM or other disease, use of such a substance in manufacture of a composition for administration, e.g. for increasing *LRP5* expression for instance in treatment of IDDM or other disease, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

A further aspect of the present invention provides a polypeptide which has the amino acid sequence shown in Figure 5(c), which may be in isolated and/or purified form, free or substantially free of material with which it is naturally associated, such as other polypeptides or such as human polypeptides other than that for which the amino acid sequence is shown in Figure 5(c), or (for example if produced by expression in a prokaryotic cell) lacking in native glycosylation, e.g. unglycosylated. Further polypeptides according to the present invention have an amino acid sequence selected from that shown in the polypeptide shown in Figure 11(c), that shown in 12(d), and the partial polypeptide shown in Figure 16(d).

Polypeptides which are amino acid sequence variants, alleles, derivatives or mutants are also provided by the present invention. A polypeptide which is a variant, allele, derivative or mutant may have an amino acid sequence which differs from that given in a figure herein by one or more of addition, substitution, deletion and insertion of one or more amino acids. Preferred such polypeptides have LRP5 function, that is to say have one or more of the following properties: immunological cross-reactivity with an antibody reactive the polypeptide for which the sequence is given in a figure herein; sharing an epitope with the polypeptide for which the amino acid sequence is shown in a figure herein (as determined for example by immunological cross-reactivity between the two polypeptides; a biological activity which is inhibited by an antibody raised against the polypeptide whose sequence is

shown in a figure herein; ability to reduce serum triglyceride; ability to reduce serum cholesterol; ability to interact with and/or reduce serum levels of very low-density lipoprotein particles; ability to affect serum alkaline phosphatase levels. Alteration of sequence may change the nature and/or level of activity and/or stability of the LRP5 protein.

A polypeptide which is an amino acid sequence variant, allele, derivative or mutant of the amino acid sequence shown in a figure herein may comprise an amino acid sequence which shares greater than about 35% sequence identity with the sequence shown, greater than about 40%, greater than about 50%, greater than about 60%, greater than about 70%, greater than about 80%, greater than about 90% or greater than about 95%. The sequence may share greater than about 60% similarity, greater than about 70% similarity, greater than about 80% similarity or greater than about 90% similarity with the amino acid sequence shown in the relevant figure. Amino acid similarity is generally defined with reference to the algorithm GAP (Genetics Computer Group, Madison, WI) as noted above, or the TBLASTN program, of Altschul et al. (1990) J. Mol. Biol. 215: 403-10. Similarity allows for "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Particular amino acid sequence variants may differ from that shown in a figure herein by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5-10, 10-20 20-30, 30-50, 50-100, 100-150, or more than 150 amino acids.

Sequence comparison may be made over the full-length of the relevant sequence shown herein, or may more preferably be over a contiguous sequence of about or greater than about 20, 25, 30, 33, 40, 50, 67, 133, 167, 200, 233, 267, 300, 333, 400, 450, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, or more amino acids or nucleotide triplets, compared with the relevant amino acid sequence or nucleotide

sequence as the case may be.

The present invention also includes active portions, fragments, derivatives and functional mimetics of the polypeptides of the invention. An "active portion" of a polypeptide means a peptide which is less than said full length polypeptide, but which retains a biological activity, such as a biological activity selected from binding to ligand, involvement in endocytosis. Thus an active portion of the LRP5 polypeptide may, in one embodiment, include the transmembrane domain and the portion of the cytoplasmic tail involved in endocytosis. Such an active fragment may be included as part of a fusion protein, e.g. including a binding portion for a different ligand. In different embodiments, combinations of LDL and EGF motifs may be included in a molecule to confer on the molecule different binding specificities.

A "fragment" of a polypeptide generally means a stretch of amino acid residues of at least about five contiguous amino acids, often at least about seven contiguous amino acids, typically at least about nine contiguous amino acids, more preferably at least about 13 contiguous amino acids, and, more preferably, at least about 20 to 30 or more contiguous amino acids. Fragments of the LRP5 polypeptide sequence may include antigenic determinants or epitopes useful for raising antibodies to a portion of the amino acid sequence. Alanine scans are commonly used to find and refine peptide motifs within polypeptides, this involving the systematic replacement of each residue in turn with the amino acid alanine, followed by an assessment of biological activity.

Preferred fragments of LRP5 include those with any of the following amino acid sequences:

SYFHLFPPPPSPCTDSS

VDGRQNIKRAKDDGT

EVLFTTGLIRPVALVVDN

IQGHLDFVMDILVFHS,

which may be used for instance in raising or isolating antibodies. Variant and derivative peptides, peptides which have an amino acid sequence which differs from one of these sequences by way of addition, insertion, deletion or

substitution of one or more amino acids are also provided by the present invention, generally with the proviso that the variant or derivative peptide is bound by an antibody or other specific binding member which binds one of the peptides whose sequence is shown. A peptide which is a variant or derivative of one of the shown peptides may compete with the shown peptide for binding to a specific binding member, such as an antibody or antigen-binding fragment thereof.

A "derivative" of a polypeptide or a fragment thereof may include a polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve one or more of insertion, addition, deletion or substitution of one or more amino acids, which may be without fundamentally altering the qualitative nature of biological activity of the wild type polypeptide. Also encompassed within the scope of the present invention are functional mimetics of active fragments of the LRP5 polypeptides provided (including alleles, mutants, derivatives and variants). The term "functional mimetic" means a substance which may not contain an active portion of the relevant amino acid sequence, and probably is not a peptide at all, but which retains in qualitative terms biological activity of natural LRP5 polypeptide. The design and screening of candidate mimetics is described in detail below.

Sequences of amino acid sequence variants representative of preferred embodiments of the present invention are shown in Table 5 and Table 6. Screening for the presence of one or more of these in a test sample has a diagnostic and/or prognostic use, for instance in determining IDDM or other disease susceptibility, as discussed below.

Other fragments of the polypeptides for which sequence information is provided herein are provided as aspects of the present invention, for instance corresponding to functional domains. One such functional domain is the putative extracellular domain, such that a polypeptide fragment according to the present invention may include the

extracellular domain of the polypeptide of which the amino acid sequence is shown in Figure 5(e) or Figure 5(c). This runs to amino acid 1385 of the precursor sequence of Figure 5(c). Another useful LRP5 domain is the cytoplasmic domain, 207 amino acids shown in Figure 5(d). This may be used in targeting proteins to move through the endocytotic pathway.

A polypeptide according to the present invention may be isolated and/or purified (e.g. using an antibody) for instance after production by expression from encoding nucleic acid (for which see below). Thus, a polypeptide may be provided free or substantially free from contaminants with which it is naturally associated (if it is a naturally-occurring polypeptide). A polypeptide may be provided free or substantially free of other polypeptides. Polypeptides according to the present invention may be generated wholly or partly by chemical synthesis. The isolated and/or purified polypeptide may be used in formulation of a composition, which may include at least one additional component, for example a pharmaceutical composition including a pharmaceutically acceptable excipient, vehicle or carrier. A composition including a polypeptide according to the invention may be used in prophylactic and/or therapeutic treatment as discussed below.

A polypeptide, peptide fragment, allele, mutant, derivative or variant according to the present invention may be used as an immunogen or otherwise in obtaining specific antibodies. Antibodies are useful in purification and other manipulation of polypeptides and peptides, diagnostic screening and therapeutic contexts. This is discussed further below.

A polypeptide according to the present invention may be used in screening for molecules which affect or modulate its activity or function, e.g. binding to ligand, involvement in endocytosis, movement from an intracellular compartment to the cell surface, movement from the cell surface to an intracellular compartment. Such molecules may interact with the ligand binding portion of LRP5, the cytoplasmic portion of

LRP5, or with one or more accessory molecules e.g. involved in movement of vesicles containing LRP5 to and from the cell surface, and may be useful in a therapeutic (possibly including prophylactic) context.

It is well known that pharmaceutical research leading to the identification of a new drug may involve the screening of very large numbers of candidate substances, both before and even after a lead compound has been found. This is one factor which makes pharmaceutical research very expensive and time-consuming. Means for assisting in the screening process can have considerable commercial importance and utility. Such means for screening for substances potentially useful in treating or preventing IDDM or other disease is provided by polypeptides according to the present invention. Substances identified as modulators of the polypeptide represent an advance in the fight against IDDM and other diseases since they provide basis for design and investigation of therapeutics for *in vivo* use. Furthermore, they may be useful in any of a number of conditions, including autoimmune diseases, such as glomerulonephritis, diseases and disorders involving disruption of endocytosis and/or antigen presentation, diseases and disorders involving cytokine clearance and/or inflammation, viral infection, pathogenic bacterial toxin contamination, elevation of free fatty acids or hypercholesterolemia, type 2 diabetes, osteoporosis, and Alzheimer's disease, given the functional indications for LRP5, discussed elsewhere herein. As noted elsewhere, LRP5, fragments thereof, and nucleic acid according to the invention may also be useful in combatting any of these diseases and disorders.

A method of screening for a substance which modulates activity of a polypeptide may include contacting one or more test substances with the polypeptide in a suitable reaction medium, testing the activity of the treated polypeptide and comparing that activity with the activity of the polypeptide in comparable reaction medium untreated with the test substance or substances. A difference in activity between the treated and untreated polypeptides is indicative of a

modulating effect of the relevant test substance or substances.

Combinatorial library technology (Schultz, JS (1996) Biotechnol. Prog. 12:729-743) provides an efficient way of testing a potentially vast number of different substances for ability to modulate activity of a polypeptide. Prior to or as well as being screened for modulation of activity, test substances may be screened for ability to interact with the polypeptide, e.g. in a yeast two-hybrid system (which requires that both the polypeptide and the test substance can be expressed in yeast from encoding nucleic acid). This may be used as a coarse screen prior to testing a substance for actual ability to modulate activity of the polypeptide.

Following identification of a substance which modulates or affects polypeptide activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

Thus, the present invention extends in various aspects not only to a substance identified as a modulator of polypeptide activity, in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a method comprising administration of such a composition to a patient, e.g. for treatment (which may include preventative treatment) of IDDM or other disease, use of such a substance in manufacture of a composition for administration, e.g. for treatment of IDDM or other disease, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

A substance identified using as a modulator of polypeptide or promoter function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many in vivo pharmaceutical uses. Accordingly, a mimetic

or mimick of the substance (particularly if a peptide) may be designed for pharmaceutical use. The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration, e.g. peptides are not well suited as active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing may be used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g. by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled to according its physical properties, e.g. stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g. spectroscopic techniques, X-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process.

In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modelled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, and

does not degrade in vivo, while retaining the biological activity of the lead compound. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Mimetics of substances identified as having ability to modulate LRP5 polypeptide or promoter activity using a screening method as disclosed herein are included within the scope of the present invention. A polypeptide, peptide or substance able to modulate activity of a polypeptide according to the present invention may be provided in a kit, e.g. sealed in a suitable container which protects its contents from the external environment. Such a kit may include instructions for use.

A convenient way of producing a polypeptide according to the present invention is to express nucleic acid encoding it, by use of the nucleic acid in an expression system. Accordingly, the present invention also encompasses a method of making a polypeptide (as disclosed), the method including expression from nucleic acid encoding the polypeptide (generally nucleic acid according to the invention). This may conveniently be achieved by growing a host cell in culture, containing such a vector, under appropriate conditions which cause or allow expression of the polypeptide. Polypeptides may also be expressed in *in vitro* systems, such as reticulocyte lysate.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, eukaryotic cells such as mammalian and yeast, and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, COS cells and many others. A common, preferred bacterial host is *E. coli*. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator

fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley & Sons, 1992.

Thus, a further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. The nucleic acid of the invention may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell.

A still further aspect provides a method which includes introducing the nucleic acid into a host cell. The introduction, which may (particularly for in vitro introduction) be generally referred to without limitation as "transformation", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed

cells) under conditions for expression of the gene, so that the encoded polypeptide is produced. If the polypeptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into the culture medium. Following production by expression, a polypeptide may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in the formulation of a composition which may include one or more additional components, such as a pharmaceutical composition which includes one or more pharmaceutically acceptable excipients, vehicles or carriers (e.g. see below).

Introduction of nucleic acid may take place *in vivo* by way of gene therapy, as discussed below. A host cell containing nucleic acid according to the present invention, e.g. as a result of introduction of the nucleic acid into the cell or into an ancestor of the cell and/or genetic alteration of the sequence endogenous to the cell or ancestor (which introduction or alteration may take place *in vivo* or *ex vivo*), may be comprised (e.g. in the soma) within an organism which is an animal, particularly a mammal, which may be human or non-human, such as rabbit, guinea pig, rat, mouse or other rodent, cat, dog, pig, sheep, goat, cattle or horse, or which is a bird, such as a chicken. Genetically modified or transgenic animals or birds comprising such a cell are also provided as further aspects of the present invention.

Thus, in various further aspects, the present invention provides a non-human animal with a human *LRP5* transgene within its genome. The transgene may have the sequence of any of the isoforms identified herein or a mutant, derivative, allele or variant thereof as disclosed. In one preferred embodiment, the heterologous human *LRP5* sequence replaces the endogenous animal sequence. In other preferred embodiments, one or more copies of the human *LRP5* sequence are added to the animal genome.

Preferably the animal is a rodent, and most preferably mouse or rat.

This may have a therapeutic aim. (Gene therapy is discussed below.) The presence of a mutant, allele or variant sequence within cells of an organism, particularly when in

place of a homologous endogenous sequence, may allow the organism to be used as a model in testing and/or studying the role of the *LRP5* gene or substances which modulate activity of the encoded polypeptide and/or promoter in vitro or are otherwise indicated to be of therapeutic potential.

An animal model for *LRP5* deficiency may be constructed using standard techniques for introducing mutations into an animal germ-line. In one example of this approach, using a mouse, a vector carrying an insertional mutation within the *LRP5* gene may be transfected into embryonic stem cells. A selectable marker, for example an antibiotic resistance gene such as neoR, may be included to facilitate selection of clones in which the mutant gene has replaced the endogenous wild type homologue. Such clones may be also be identified or further investigated by Southern blot hybridisation. The clones may then be expanded and cells injected into mouse blastocyst stage embryos. Mice in which the injected cells have contributed to the development of the mouse may be identified by Southern blotting. These chimeric mice may then be bred to produce mice which carry one copy of the mutation in the germ line. These heterozygous mutant animals may then be bred to produce mice carrying mutations in the gene homozygously. The mice having a heterozygous mutation in the *LRP5* gene may be a suitable model for human individuals having one copy of the gene mutated in the germ line who are at risk of developing IDDM or other disease.

Animal models may also be useful for any of the various diseases discussed elsewhere herein.

Instead of or as well as being used for the production of a polypeptide encoded by a transgene, host cells may be used as a nucleic acid factory to replicate the nucleic acid of interest in order to generate large amounts of it. Multiple copies of nucleic acid of interest may be made within a cell when coupled to an amplifiable gene such as dihyrofolate reductase (DHFR), as is well known. Host cells transformed with nucleic acid of interest, or which are descended from host cells into which nucleic acid was introduced, may be cultured under suitable conditions, e.g. in a fermentor, taken

from the culture and subjected to processing to purifiy the nucleic acid. Following purification, the nucleic acid or one or more fragments thereof may be used as desired, for instance in a diagnostic or prognostic assay as discussed elsewhere herein.

The provision of the novel LRP-5 polypeptide isoforms and mutants, alleles, variants and derivatives enables for the first time the production of antibodies able to bind these molecules specifically.

Accordingly, a further aspect of the present invention provides an antibody able to bind specifically to the polypeptide whose sequence is given in a figure herein. Such an antibody may be specific in the sense of being able to distinguish between the polypeptide it is able to bind and other human polypeptides for which it has no or substantially no binding affinity (e.g. a binding affinity of about 1000x less). Specific antibodies bind an epitope on the molecule which is either not present or is not accessible on other molecules. Antibodies according to the present invention may be specific for the wild-type polypeptide. Antibodies according to the invention may be specific for a particular mutant, variant, allele or derivative polypeptide as between that molecule and the wild-type polypeptide, so as to be useful in diagnostic and prognostic methods as discussed below. Antibodies are also useful in purifying the polypeptide or polypeptides to which they bind, e.g. following production by recombinant expression from encoding nucleic acid.

Preferred antibodies according to the invention are isolated, in the sense of being free from contaminants such as antibodies able to bind other polypeptides and/or free of serum components. Monoclonal antibodies are preferred for some purposes, though polyclonal antibodies are within the scope of the present invention.

Antibodies may be obtained using techniques which are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof.

Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al., 1992, Nature 357: 80-82). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest.

Suitable peptides for use in immunising an animal and/or isolating anti-LRP5 antibody include any of the following amino acid sequences:

SYFHLFPPPPSPCTDSS

VDGRQNIKRAKDDGT

EVLFTTGLIRPVALVVDN

IQGHLDFVMDILVFHS.

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus the invention covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic molecules and molecules whose shape mimicks that of an antibody enabling it to bind an antigen or epitope.

Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of

the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')2 fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

A hybridoma producing a monoclonal antibody according to the present invention may be subject to genetic mutation or other changes. It will further be understood by those skilled in the art that a monoclonal antibody can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP184187A, GB 2188638A or EP-A-0239400. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

The reactivities of antibodies on a sample may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

One favoured mode is by covalent linkage of each antibody

with an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge. Particular embodiments of antibodies according to the present invention include antibodies able to bind and/or which bind specifically, e.g. with an affinity of at least 10^{-7} M, to one of the following peptides:

SYFHLFPPPPSPCTDSS

VDGRQNIKRAKDDGT

EVLFTTGLIRPVALVVDN

IQGHLDFVMDILVFHS.

Antibodies according to the present invention may be used in screening for the presence of a polypeptide, for example in a test sample containing cells or cell lysate as discussed, and may be used in purifying and/or isolating a polypeptide according to the present invention, for instance following production of the polypeptide by expression from encoding nucleic acid therefor. Antibodies may modulate the activity of the polypeptide to which they bind and so, if that

polypeptide has a deleterious effect in an individual, may be useful in a therapeutic context (which may include prophylaxis).

An antibody may be provided in a kit, which may include instructions for use of the antibody, e.g. in determining the presence of a particular substance in a test sample. One or more other reagents may be included, such as labelling molecules, buffer solutions, elutants and so on. Reagents may be provided within containers which protect them from the external environment, such as a sealed vial.

The identification of the *LRP5* gene and indications of its association with IDDM and other diseases paves the way for aspects of the present invention to provide the use of materials and methods, such as are disclosed and discussed above, for establishing the presence or absence in a test sample of an variant form of the gene, in particular an allele or variant specifically associated with IDDM or other disease. This may be for diagnosing a predisposition of an individual to IDDM or other disease. It may be for diagnosing IDDM of a patient with the disease as being associated with the IDDM4 gene.

This allows for planning of appropriate therapeutic and/or prophylactic treatment, permitting stream-lining of treatment by targeting those most likely to benefit.

A variant form of the gene may contain one or more insertions, deletions, substitutions and/or additions of one or more nucleotides compared with the wild-type sequence (such as shown in Table 5 or Table 6) which may or may not disrupt the gene function. Differences at the nucleic acid level are not necessarily reflected by a difference in the amino acid sequence of the encoded polypeptide. However, a mutation or other difference in a gene may result in a frame-shift or stop codon, which could seriously affect the nature of the polypeptide produced (if any), or a point mutation or gross mutational change to the encoded polypeptide, including insertion, deletion, substitution and/or addition of one or more amino acids or regions in the polypeptide. A mutation in a promoter sequence or other regulatory region may prevent or reduce expression from the gene or affect the processing or

stability of the mRNA transcript. For instance, a sequence alteration may affect alternative splicing of mRNA. As discussed, various *LRP5* isoforms resulting from alternative splicing are provided by the present invention.

There are various methods for determining the presence or absence in a test sample of a particular nucleic acid sequence, such as the sequence shown in any figure herein, or a mutant, variant or allele thereof, e.g. including an alteration shown in Table 5 or Table 6.

Tests may be carried out on preparations containing genomic DNA, cDNA and/or mRNA. Testing cDNA or mRNA has the advantage of the complexity of the nucleic acid being reduced by the absence of intron sequences, but the possible disadvantage of extra time and effort being required in making the preparations. RNA is more difficult to manipulate than DNA because of the wide-spread occurrence of RN'ases. Nucleic acid in a test sample may be sequenced and the sequence compared with the sequence shown in any of the figures herein, to determine whether or not a difference is present. If so, the difference can be compared with known susceptibility alleles (e.g. as shown in Table 5 or Table 6) to determine whether the test nucleic acid contains one or more of the variations indicated, or the difference can be investigated for association with IDDM or other disease.

Since it will not generally be time- or labour-efficient to sequence all nucleic acid in a test sample or even the whole *LRP5* gene, a specific amplification reaction such as PCR using one or more pairs of primers may be employed to amplify the region of interest in the nucleic acid, for instance the *LRP5* gene or a particular region in which polymorphisms associated with IDDM or other disease susceptibility occur. The amplified nucleic acid may then be sequenced as above, and/or tested in any other way to determine the presence or absence of a particular feature. Nucleic acid for testing may be prepared from nucleic acid removed from cells or in a library using a variety of other techniques such as restriction enzyme digest and electrophoresis.

Nucleic acid may be screened using a variant- or allele-specific probe. Such a probe corresponds in sequence to a

region of the *LRP5* gene, or its complement, containing a sequence alteration known to be associated with IDDM or other disease susceptibility. Under suitably stringent conditions, specific hybridisation of such a probe to test nucleic acid is indicative of the presence of the sequence alteration in the test nucleic acid. For efficient screening purposes, more than one probe may be used on the same test sample.

Allele- or variant-specific oligonucleotides may similarly be used in PCR to specifically amplify particular sequences if present in a test sample. Assessment of whether a PCR band contains a gene variant may be carried out in a number of ways familiar to those skilled in the art. The PCR product may for instance be treated in a way that enables one to display the polymorphism on a denaturing polyacrylamide DNA sequencing gel, with specific bands that are linked to the gene variants being selected.

SSCP heteroduplex analysis may be used for screening DNA fragments for sequence variants/mutations. It generally involves amplifying radiolabelled 100-300 bp fragments of the gene, diluting these products and denaturing at 95°C. The fragments are quick-cooled on ice so that the DNA remains in single stranded form. These single stranded fragments are run through acrylamide based gels. Differences in the sequence composition will cause the single stranded molecules to adopt difference conformations in this gel matrix making their mobility different from wild type fragments, thus allowing detecting of mutations in the fragments being analysed relative to a control fragment upon exposure of the gel to X-ray film. Fragments with altered mobility/conformations may be directly excised from the gel and directly sequenced for mutation.

Sequencing of a PCR product may involve precipitation with isopropanol, resuspension and sequencing using a TaqFS+ Dye terminator sequencing kit. Extension products may be electrophoresed on an ABI 377 DNA sequencer and data analysed using Sequence Navigator software.

A further possible screening approach employs a PTT assay in which fragments are amplified with primers that contain the consensus Kozak initiation sequences and a T7 RNA

polymerase promoter. These extra sequences are incorporated into the 5' primer such that they are in frame with the native coding sequence of the fragment being analysed. These PCR products are introduced into a coupled transcription/translation system. This reaction allows the production of RNA from the fragment and translation of this RNA into a protein fragment. PCR products from controls make a protein product of a wild type size relative to the size of the fragment being analysed. If the PCR product analysed has a frame-shift or nonsense mutation, the assay will yield a truncated protein product relative to controls. The size of the truncated product is related to the position of the mutation, and the relative region of the gene from this patient may be sequenced to identify the truncating mutation.

An alternative or supplement to looking for the presence of variant sequences in a test sample is to look for the presence of the normal sequence, e.g. using a suitably specific oligonucleotide probe or primer. Use of oligonucleotide probes and primers has been discussed in more detail above.

Allele- or variant-specific oligonucleotide probes or primers according to embodiments of the present invention may be selected from those shown in Table 4, Table 7 or Table 8.

Approaches which rely on hybridisation between a probe and test nucleic acid and subsequent detection of a mismatch may be employed. Under appropriate conditions (temperature, pH etc.), an oligonucleotide probe will hybridise with a sequence which is not entirely complementary. The degree of base-pairing between the two molecules will be sufficient for them to anneal despite a mis-match. Various approaches are well known in the art for detecting the presence of a mis-match between two annealing nucleic acid molecules.

For instance, RN'ase A cleaves at the site of a mis-match. Cleavage can be detected by electrophoresing test nucleic acid to which the relevant probe or probe has annealed and looking for smaller molecules (i.e. molecules with higher electrophoretic mobility) than the full length probe/test hybrid.

Thus, an oligonucleotide probe that has the sequence of a

region of the normal *LRP5* gene (either sense or anti-sense strand) in which mutations associated with IDDM or other disease susceptibility are known to occur (e.g. see Table 5 and Table 6) may be annealed to test nucleic acid and the presence or absence of a mis-match determined. Detection of the presence of a mis-match may indicate the presence in the test nucleic acid of a mutation associated with IDDM or other disease susceptibility. On the other hand, an oligonucleotide probe that has the sequence of a region of the gene including a mutation associated with IDDM or other disease susceptibility may be annealed to test nucleic acid and the presence or absence of a mis-match determined. The presence of a mis-match may indicate that the nucleic acid in the test sample has the normal sequence (the absence of a mis-match indicating that the test nucleic acid has the mutation). In either case, a battery of probes to different regions of the gene may be employed.

The presence of differences in sequence of nucleic acid molecules may be detected by means of restriction enzyme digestion, such as in a method of DNA fingerprinting where the restriction pattern produced when one or more restriction enzymes are used to cut a sample of nucleic acid is compared with the pattern obtained when a sample containing the normal gene shown in a figure herein or a variant or allele, e.g. as containing an alteration shown in Table 5 or Table 6 is digested with the same enzyme or enzymes.

The presence or absence of a lesion in a promoter or other regulatory sequence may also be assessed by determining the level of mRNA production by transcription or the level of polypeptide production by translation from the mRNA. Determination of promoter activity has been discussed above.

A test sample of nucleic acid may be provided for example by extracting nucleic acid from cells or biological tissues or fluids, urine, saliva, faeces, a buccal swab, biopsy or preferably blood, or for pre-natal testing from the amnion, placenta or foetus itself.

There are various methods for determining the presence or

absence in a test sample of a particular polypeptide, such as the polypeptide with the amino acid sequence shown in any figure herein or an amino acid sequence mutant, variant or allele thereof.

A sample may be tested for the presence of a binding partner for a specific binding member such as an antibody (or mixture of antibodies), specific for one or more particular variants of the polypeptide shown in a figure herein. A sample may be tested for the presence of a binding partner for a specific binding member such as an antibody (or mixture of antibodies), specific for the polypeptide shown in a figure herein. In such cases, the sample may be tested by being contacted with a specific binding member such as an antibody under appropriate conditions for specific binding, before binding is determined, for instance using a reporter system as discussed. Where a panel of antibodies is used, different reporting labels may be employed for each antibody so that binding of each can be determined.

A specific binding member such as an antibody may be used to isolate and/or purify its binding partner polypeptide from a test sample, to allow for sequence and/or biochemical analysis of the polypeptide to determine whether it has the sequence and/or properties of the polypeptide whose sequence is disclosed herein, or if it is a mutant or variant form. Amino acid sequence is routine in the art using automated sequencing machines.

A test sample containing one or more polypeptides may be provided for example as a crude or partially purified cell or cell lysate preparation, e.g. using tissues or cells, such as from saliva, faeces, or preferably blood, or for pre-natal testing from the amnion, placenta or foetus itself.

Whether it is a polypeptide, antibody, peptide, nucleic acid molecule, small molecule or other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis

may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practioners and other medical doctors.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may include, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, or Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibody or cell specific ligands. Targeting may be desirable for a variety of reasons; for example if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

Instead of administering an agent directly, it may be be produced in target cells by expression from an encoding gene introduced into the cells, e.g. in a viral vector (see below). The vector may be targeted to the specific cells to be treated, or it may contain regulatory elements which are switched on more or less selectively by the target cells. Viral vectors may be targeted using specific binding molecules, such as a sugar, glycolipid or protein such as an antibody or binding fragment thereof. Nucleic acid may be targeted by means of linkage to a protein ligand (such as an antibody or binding fragment thereof) via polylysine, with the ligand being specific for a receptor present on the surface of the target cells.

An agent may be administered in a precursor form, for conversion to an active form by an activating agent produced in, or targeted to, the cells to be treated. This type of approach is sometimes known as ADEPT or VDEPT; the former involving targeting the activating agent to the cells by conjugation to a cell-specific antibody, while the latter involves producing the activating agent, e.g. an enzyme, in a vector by expression from encoding DNA in a viral vector (see for example, EP-A-415731 and WO 90/07936).

Nucleic acid according to the present invention, e.g. encoding the authentic biologically active LRP-5 polypeptide or a functional fragment thereof, may be used in a method of gene therapy, to treat a patient who is unable to synthesize the active polypeptide or unable to synthesize it at the normal level, thereby providing the effect provided by the wild-type with the aim of treating and/or preventing one or more symptoms of IDDM and/or one or more other diseases.

Vectors such as viral vectors have been used to introduce

genes into a wide variety of different target cells. Typically the vectors are exposed to the target cells so that transfection can take place in a sufficient proportion of the cells to provide a useful therapeutic or prophylactic effect from the expression of the desired polypeptide. The transfected nucleic acid may be permanently incorporated into the genome of each of the targeted cells, providing long lasting effect, or alternatively the treatment may have to be repeated periodically.

A variety of vectors, both viral vectors and plasmid vectors, are known in the art, see e.g. US Patent No. 5,252,479 and WO 93/07282. In particular, a number of viruses have been used as gene transfer vectors, including adenovirus, papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV, and retroviruses, including gibbon ape leukaemia virus, Rous Sarcoma Virus, Venezualian equine enchephalitis virus, Moloney murine leukaemia virus and murine mammary tumourvirus. Many gene therapy protocols in the prior art have used disabled murine retroviruses.

Disabled virus vectors are produced in helper cell lines in which genes required for production of infectious viral particles are expressed. Helper cell lines are generally missing a sequence which is recognised by the mechanism which packages the viral genome and produce virions which contain no nucleic acid. A viral vector which contains an intact packaging signal along with the gene or other sequence to be delivered (e.g. encoding the LRP5 polypeptide or a fragment thereof) is packaged in the helper cells into infectious virion particles, which may then be used for the gene delivery.

Other known methods of introducing nucleic acid into cells include electroporation, calcium phosphate co-precipitation, mechanical techniques such as microinjection, transfer mediated by liposomes and direct DNA uptake and receptor-mediated DNA transfer. Liposomes can encapsulate RNA, DNA and virions for delivery to cells. Depending on factors such as pH, ionic strength and divalent cations being present, the composition of liposomes may be tailored for targeting of particular cells or tissues. Liposomes include phospholipids

and may include lipids and steroids and the composition of each such component may be altered. Targeting of liposomes may also be achieved using a specific binding pair member such as an antibody or binding fragment thereof, a sugar or a glycolipid.

The aim of gene therapy using nucleic acid encoding the polypeptide, or an active portion thereof, is to increase the amount of the expression product of the nucleic acid in cells in which the level of the wild-type polypeptide is absent or present only at reduced levels. Such treatment may be therapeutic or prophylactic, particularly in the treatment of individuals known through screening or testing to have an IDDM4 susceptibility allele and hence a predisposition to the disease.

Similar techiques may be used for anti-sense regulation of gene expression, e.g. targeting an antisense nucleic acid molecule to cells in which a mutant form of the gene is expressed, the aim being to reduce production of the mutant gene product. Other approaches to specific down-regulation of genes are well known, including the use of ribozymes designed to cleave specific nucleic acid sequences. Ribozymes are nuceic acid molecules, actually RNA, which specifically cleave single-stranded RNA, such as mRNA, at defined sequences, and their specificity can be engineered. Hammerhead ribozymes may be preferred because they recognise base sequences of about 11-18 bases in length, and so have greater specificity than ribozymes of the Tetrahymena type which recognise sequences of about 4 bases in length, though the latter type of ribozymes are useful in certain circumstances. References on the use of ribozymes include Marschall, et al. Cellular and Molecular Neurobiology, 1994. 14(5): 523; Hasselhoff, Nature 334: 585 (1988) and Cech, J. Amer. Med. Assn., 260: 3030 (1988).

Aspects of the present invention will now be illustrated with reference to the accompanying figures described already above and experimental exemplification, by way of example and not limitation. Further aspects and embodiments will be apparent to those of ordinary skill in the art. All documents mentioned in this specification are hereby incorporated herein

by reference.

EXAMPLE 1

CLONING OF *LRP5*

As noted above, confirmation of linkage to two of the 18 potential loci for IDDM predisposition was achieved by analysis of two family sets (102 UK families and 84 USA families), *IDDM4* on chromosome 11q13 (MLS 1.3 , P = 0.01 at *FGF3*) and *IDDM5* on chromosome 6q (MLS 1.8 P= 0.003 at *ESR*). At *IDDM4* the most significant linkage was obtained in the subset of families sharing 1 or 0 alleles IBD at HLA (MLS = 2.8; P=0.0002; ls = 1.2) (Davies et al, 1994). This linkage was also observed by Hashimoto et al (1994) using 251 affected sibpairs, obtaining P= 0.0008 in all sibpairs. Combining these results, with 596 families, provides substantial support for *IDDM4* ($P = 1.5 \times 10^{-6}$) (Todd and Farrall, 1996; Luo et al, 1996).

Multipoint analysis with other markers in the *FGF3* region produced an MLS of 2.3 at *FGF3* and *D11S1883* (ls = 1.19), and delineated the interval to a 27cM region, flanked by the markers *D11S903* and *D11S527* (Figure 1).

Multipoint linkage analysis cannot localise the gene to a small region unless several thousand multiplex families are available. Instead, association mapping has been used for rare single gene diseases which can narrow the interval containing the disease gene to less than 2cM or 2M bases. Nevertheless, this method is highly unpredictable and has not previously been used to locate a polygene for a common disease. Association mapping has been used to locate the *IDDM2/INS* polygene but this relied on the selection of a functional candidate polymorphism/gene and was restricted to a very small (<30kb) region. Linkage disequilibrium (LD) or association studies were carried out in order to delineate the *IDDM4* region to less than 2cM. In theory, association of a particular allele very close to the founder mutation will be detected in populations descended from that founder. The transmission disequilibrium test (TDT, Spielman et al, 1993) measures association by assessing the deviation from 50% of

the transmission of alleles from a marker locus from parents to affected children. The detection of association is dependent on the ancestry of each population studied to be as homogeneous as possible, in order to reduce the possiblity that the presence of several founder-chromosomes, decreasing the power to detect the association. These parameters are highly unpredictable.

Analysis of markers spanning the *IDDM4* linkage interval, LD was detected at *D11S1917(UT5620)* in 554 families, P=0.01. A physical map of this region, comprising approximately 500kb, was achieved by constructing a pac, bac and cosmid contig (Figure 2). The region was physically mapped by hybridisation of markers onto restriction-enzyme digested clones resolved through agarose, and Southern blotted.

Further microsatellites (both published, and those isolated from the clones by microsatellite rescue) were analysed within 1289 families, from four different populations (UK, USA, Sardinia and Norway). A LD graph was constructed, with a peak at *H0570POLYA*, P = 0.001, flanked by the markers *D11S987* and *18018AC* (Figure 3). The LD detected at a polymorphic marker is influenced by allele frequency, and whether the mutation causing susceptibility to type 1 diabetes arose on a chromosome where the allele in LD is the same allele as that on protective or neutral chromosomes. In the case where the marker being analysed has the same allele in LD with both susceptible and protective genotypes, these will remain undetected by single point analysis, in effect cancelling each other out, and showing little or no evidence for LD with the disease locus. Unpredictability of the method arising from this has been noted already above.

In order to maximise the information obtained with each marker, a three point rolling LD curve was produced with the *IDDM4* markers (Figure 4). In this case the percentage transmission (%T) was calculated from a marker, and its two immediate flanking markers, and averaged between them to minimise the effects of fluctuating allele frequency. This also produced a peak at *H0570POLYA*, with P=0.04, and indicates that the *IDDM4* mutation is more likely to be in the interval *E0864CA - D11S1337* (75kb).

By the identification of this 75kb interval which shows association with type 1 diabetes, disease associated haplotypes were identified. These are derived from the original founder chromosomes on which the diabetes mutation or mutations *IDDM4* arose. In order to identify the mutation causing susceptibility to type 1 diabetes, a refined linkage disequilibrium curve, based on single nucleotide polymorphisms (SNPs) and haplotypes, is constructed. SNPs are identified by sequencing individuals with specific haplotypes which have been identified from the microsatellite analysis: homozygous susceptible to type 1 diabetes, homozygous protective for type 1 diabetes, and controls. One of these SNPs may be the etiological mutation *IDDM4*, or may be in very strong linkage disequilibrium with the primary disease locus, and hence be at a peak of the refined curve. Cross-match analysis further reduces the number of candidate SNPs, as shown by the localisation of the *IDDM2* mutation by this method (Bennett et al, 1995; Bennett and Todd, 1996). This requires identification of distinct haplotypes or founder chromosomes, which have a different arrangement of alleles from the main susceptible or protective haplotypes, so that association or transmission of candidate SNP alleles can be tested in different haplotype backgrounds. The candidate mutations can be assessed for effects on gene function or regulation.

In different populations different *IDDM4* mutations may have arisen in the same gene. We are sequencing several putative founder chromosome or disease associated haplotypes from several unrelated individuals from different populations to identify candidate mutations for *IDDM4*, and which cluster in the same gene.

To carry out an extensive search for DNA mutations or polymorphisms, the entire region and flanking regions of the associated region was sequenced (the 75kb core region and 125 kb of flanking DNA). The DNA sequence also aids in gene identification and is complementary to other methods of gene identification such as cDNA selection or gene identification by DNA sequencing and comparative analysis of homologous mouse genomic DNA.

Various strategies were used in the hope of identifying

potential coding sequences within this region: sequencing, computer prediction of putative exons and promoters, and cDNA selection, to try to increase the likelihood of identifying all the genes within this interval.

Construction of Libraries for Shotgun Sequencing

DNA was prepared from either cosmids, BACs (Bacterial Artificial Chromosomes), or PACs (P1 Artificial Chromosomes). Cells containing the vector were streaked on Luria-Bertani (LB) agar plates supplemented with the appropriate antibiotic. A single colony was used to inoculate 200 ml of LB media supplemented with the appropriate antibiotic and grown overnight at 37°C. The cells were pelleted by centrifugation and plasmid DNA was prepared by following the QIAGEN (Chatsworth, CA) Tip500 Maxi plasmid/cosmid purification protocol with the following modifications; the cells from 100 ml of culture were used for each Tip500 column, the NaCl concentration of the elution buffer was increased from 1.25M to 1.7M, and the elution buffer was heated to 65°C.

Purified BAC and PAC DNA was digested with Not I restriction endonuclease and then subjected to pulse field gel electrophoresis using a BioRad CHEF Mapper system. (Richmond, CA). The digested DNA was electrophoresed overnight in a 1% low melting temperature agarose (BioRad, Richmond CA) gel that was prepared with 0.5X Tris Borate EDTA (10X stock solution, Fisher, Pittsburg, PA). The CHEF Mapper autoalgorithm default settings were used for switching times and voltages. Following electrophoresis the gel was stained with ethidium bromide (Sigma, St. Louis, MO) and visualized with a ultraviolet transilluminator. The insert band(s) was excised from the gel. The DNA was eluted from the gel slice by beta-Agarase (New England Biolabs, Beverly MA) digestion according to the manufacturer's instructions. The solution containing the DNA and digested agarose was brought to 50 mM Tris pH 8.0, 15 mM MgCl2, and 25% glycerol in a volume of 2 ml and placed in a AERO-MIST nebulizer (CIS-US, Bedford MA). The nebulizer was attatched to a nitrogen gas source and the DNA was randomly sheared at 10 psi for 30 sec. The sheared DNA was ethanol precipitated and resuspended in TE (10 mM Tris, 1

mM EDTA). The ends were made blunt by treatment with Mung Bean Nuclease (Promega, Madison, WI) at 30°C for 30 min, followed by phenol/chloroform extraction, and treatment with T4 DNA polymerase (GIBCO/BRL, Gaithersburg, MD) in multicore buffer (Promega, Madison, WI) in the presence of 40 uM dNTPs at 16°C. To facilitate subcloning of the DNA fragments, BstX I adapters (Invitrogen, Carlsbad, CA) were ligated to the fragments at 14°C overnight with T4 DNA ligase (Promega, Madison WI). Adapters and DNA fragments less than 500 bp were removed by column chromatography using a cDNA sizing column (GIBCO/BRL, Gaithersburg, MD) according to the instructions provided by the manufacturer. Fractions containing DNA greater than 1 kb were pooled and concentrated by ethanol precipitation. The DNA fragments containing BstX I adapters were ligated into the BstX I sites of pSHOT II which was constructed by subcloning the BstX I sites from pcDNA II (Invitrogen, Carlsbad, CA) into the BssH II sites of pBlueScript (Stratagene, La Jolla, CA). pSHOT II was prepared by digestion with BstX I restriction endonuclease and purified by agarose gel electrophoresis. The gel purified vector DNA was extracted from the agarose by following the Prep-A-Gene (BioRad, Richmond, CA) protocol. To reduce ligation of the vector to itself, the digested vector was treated with calf intestinal phosphatase (GIBCO/BRL, Gaithersburg, MD). Ligation reactions of the DNA fragments with the cloning vector were transformed into ultra-competent XL-2 Blue cells (Stratagene, La Jolla, CA), and plated on LB agar plates supplemented with 100 ug/ml ampicillin. Individual colonies were picked into a 96 well plate containing 100 ul/well of LB broth supplemented with ampicillin and grown overnight at 37°C. Approximately 25 ul of 80% sterile glycerol was added to each well and the cultures stored at -80°C.

Preparation of plasmid DNA

Glycerol stocks were used to inoculate 5 ml of LB broth supplemented with 100 ug/ml ampicillin either manually or by using a Tecan Genesis RSP 150 robot (Tecan AG, Hombrechtikon, Switzerland) programmed to inoculate 96 tubes containing 5 ml broth from the 96 wells. The cultures were grown overnight at

37°C with shaking to provide aeration. Bacterial cells were pelleted by centrifugation, the supernatant decanted, and the cell pellet stored at -20°C. Plasmid DNA was prepared with a QIAGEN Bio Robot 9600 (QIAGEN, Chatsworth CA) according to the Qiawell Ultra protocol. To test the frequency and size of inserts plasmid DNA was digested with the restriction endonuclease Pvu II. The size of the restriction endonuclease products was examined by agarose gel electrophoresis with the average insert size being 1 to 2 kb.

DNA Sequence Analysis of Shotgun clones

DNA sequence analysis was performed using the ABI PRISM™ dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS (Perkin Elmer, Norwalk, CT). DNA sequence analysis was performed with M13 forward and reverse primers. Following amplification in a Perkin-Elmer 9600 the extension products were purified and analyzed on an ABI PRISM 377 automated sequencer (Perkin Elmer, Norwalk, CT). Approximately 12 to 15 sequencing reactions were performed per kb of DNA to be examined e.g. 1500 reactions would be performed for a PAC insert of 100 kb.

Assembly of DNA sequences

Phred/Phrap was used for DNA sequences assembly. This program was developed by Dr. Phil Green and licensed from the University of Washington (Seattle, WA). Phred/Phrap consists of the following programs: Phred for base-calling, Phrap for sequence assembly, Crossmatch for sequence comparisons, Consed and Phrapview for visualization of data, and Repeatmasker for screening repetitive sequences. Vector and *E. coli* DNA sequences were identified by Crossmatch and removed from the DNA sequence assembly process. DNA sequence assembly was on a SUN Enterprise 4000 server running Solaris 2.51 operating system (Sun Microsystems Inc., Mountain View, CA) using default Phrap parameters. The sequence assemblies were further analyzed using Consed and Phrapview.

BioInformatic Analysis of Assembled DNA Sequences

When the assembled DNA sequences approached five to six

fold coverage of the region of interest the exon and promoter prediction abilities of the program GRAIL (ApoCom, Oak Ridge) were utilized to aid in gene identification. ApoCom GRAIL is a commercial version of the Department of Energy developed GRAIL Gene Characterization Software licensed to ApoCom Inc. by Lockheed Martin Energy Research Corporation and ApoCom Client Tool for Genomics (ACTG) TM.

The DNA sequences at various stages of assembly were queried against the DNA sequences in the GenBank database (subject) using the BLAST algorithm (S.F. Altschul, *et al.* (1990) J. Mol. Biol. 215, 403-410), with default parameters. When examining large contiguous sequences of DNA repetitive elements were masked following identification by crossmatch with a database of mammalian repetitive elements. Following BLAST analysis the results were compiled by a parser program written by Dr. Guochun Xie (Merck Research Lab). The parser provided the following information from the database for each DNA sequence having a similarity with a P value greater than 10^{-6}; the annotated name of the sequence, the database from which it was derived, the length and percent identity of the region of similarity, and the location of the similarity in both the query and the subject.

The BLAST analysis identified a high degree of similarities (90-100% identical) over a length of greater than 100 bp between DNA sequences we obtained and a number of human EST sequences present in the database. These human EST sequences clustered into groups that are represented by accession numbers; R73322, R50627, F07016. In general, each EST cluster is presumed to represent a single gene. The DNA sequences in R73322 cluster of 424 nucleotides had a lower but significant degree of DNA sequence similarity to the gene encoding the LDL receptor related protein (GenBank accession number X13916) and several other members of the LDL receptor family. Therefore it was concluded that the sequences that were highly similar to EST R73322 encoded a member of the LDL receptor family.

Members of each EST cluster were assembled using the program Sequencher (Perkin Elmer, Norwalk CT). To increase

the accuracy of the EST sequence data extracted from the database relevent chromatogram trace files from the genomic DNA sequences obtained from shotgun sequencing were included in the assembly. The corrected EST sequences were reanalyzed by BLAST and BLASTX. For EST cluster 3, represented by accession number R50627 analysis of the edited EST assembly revealed that this cluster was similar to members of the LDL receptor family. This result suggested the possibility that these two EST clusters were components of the same gene.

Experimentally derived cDNA sequences were assembled using the program Sequencher (Perkin Elmer, Norwalk CT). Genomic DNA sequences and cDNA sequences were compared by using the program Crossmatch which allowed for a rapid and sensitive detection of the location of exons. The identification of intron/exon boundaries was then accomplished by manually comparing the genomic and cDNA sequences by using the program GeneWorks (Intelligenetics Inc., Campbell CA).

Northern Blot Analysis

Primers 256F and 622R (Table 2) were used to amplify a PCR product of 366 bp from a fetal brain cDNA library. This product was purified on an agarose gel, the DNA extracted, and subcloned into pCR2.1 (Invitrogen, Carlsbad, CA) . The 366 bp probe was labeled by random priming with the Amersham Rediprime kit (Arlington Heights, IL) in the presence of 50-100 uCi of 3000 Ci/mmole [alpha ^{32}P]dCTP (Dupont/NEN, Boston, MA). Unincorporated nucleotides were removed with a ProbeQuant G-50 spin column (Pharmacia/ Biotech, Piscataway, NJ). The radiolabeled probe at a concentration of greater than 1 x 10^6 cpm/ml in rapid hybridization buffer (Clontech, Palo Alto, CA) was incubated overnight at 65°C with human multiple tissue Northern's I and II (Clontech, Palo Alto, CA). The blots were washed by two 15 min incubations in 2X SSC, 0.1% SDS (prepared from 20X SSC and 20 % SDS stock solutions, Fisher, Pittsburg, PA) at room temperature, followed by two 15 min incubations in 1X SSC, 0.1% SDS at room temperature, and two 30 min incubations in 0.1X SSC, 0.1% SDS at 60°C. Autoradiography of the blots was done to visualize the bands

that specifically hybridized to the radiolabeled probe.

The probe hybridized to an approximately 5-5.5 kb mRNA transcript that is most highly expressed in placenta, liver, pancreas, and prostate. It is expressed at an intermediate level in lung, skeletal muscle, kidney, spleen, thymus, ovary, small intestine, and colon. The message is expressed at a low level in brain, testis, and leukocytes. In tissues where the transcript is highly expressed, e.g. liver and pancreas, additional bands of 7kb and 1.3 kb are observed.

Isolation of full length cDNAs

PCR based techniques were used to extend regions that were highly similar to ESTs and regions identified by exon prediction software (GRAIL). The one technique utilized is a variation on Rapid Amplification of cDNA Ends (RACE) termed Reduced Complexity cDNA Analysis (RCCA) similar procedures are reported by Munroe *et .al.* (1995) PNAS 92: 2209-2213 and Wilfinger *et. al.* (1997) BioTechniques 22: 481-486. This technique relies upon a PCR template that is a pool of approximately 20,000 cDNA clones, this reduces the complexity of the template and increases the probability of obtaining longer PCR extensions. A second technique that was used to extend cDNAs was PCR between regions that were identified in the genomic sequence of having the potential to be portions of a gene e.g. sequences that were very similar to ESTs or sequences that were identified by GRAIL. These PCR reactions were done on cDNA prepared from approximately 5 ug of mRNA (Clontech, Palo Alto, CA) with the SuperScript™ choice system (Gibco/BRL, Gaithersburg, MD). The first strand cDNA synthesis was primed using 1 ug of oligo$(dT)_{12-18}$ primer and 25 ng of random hexamers per reaction. Second strand cDNA synthesis was performed according to the manufacturer's instructions.

Identification of additional exons related to EST cluster 1

We scanned 96 wells of a human fetal brain plasmid library, 20,000 clones per well, by amplifying a 366 bp PCR product using primers 256F and 622R. The reaction mix

consisted of 4 ul of plasmid DNA (0.2 ng/ml), 10 mM Tris-HCl pH 8.3, 50 mM KCl, 10% sucrose, 2.5 mM $MgCl_2$, 0.1% Tetrazine, 200 mM dNTP's, 100 ng of each primer and 0.1 ul of Taq Gold (Perkin-Elmer, Norwalk, CT). A total reaction volume of 11 ul was incubated at 95°C for 12 min followed by 32 cycles of 95°C for 30 sec, 60°C, for 30 sec and 72°C for 30 sec. Approximately 20 wells were found to contain the correct 366 bp fragment by PCR analysis. 5' and 3' RACE was subsequently performed on several of the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers, PBS 543R and PBS 873F were both used in combination with gene specific primers 117F and 518R because the orientation of the insert was not known. PCR amplification conditions consisted of 1X TaKaRa Buffer LA, 2.5 mM $MgCl_2$, 500 mM dNTP's, 0.2 ul of TaKaRa LA Taq Polymerase (PanVera, Madison WI), 100 ng of each primer and 5 ul of the plasmid library at 0.2 ng/ml. In a total reaction volume of 20 ml, the thermal cycling conditions were as follows: 92°C for 30 sec, followed by 32 cycles of 92°C for 30 sec, 1 min at 60°C and 10 min at 68°C. After the initial PCR amplification, a nested or semi-nested PCR reaction was performed using nested vector primers PBS 578R and PBS 838F and various gene specific primers (256F, 343F, 623R and 657R). The PCR products were separated from the unincorporated dNTP's and primers using QIAGEN, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 ul of water. The amplification conditions for the nested and semi-nested PCR were the same as the initial PCR amplification except that 3 ul of the purified PCR fragment was used as template and that the cycling conditions were for only 20 cycles. Products obtained from this PCR amplification were analyzed on 1% agarose gels, excised fragments were purified using QIAGEN QIAquick spin columns and sequenced using ABI dye-terminator sequencing kits. The products were analyzed on ABI 377 sequencers according to standard protocols.

Connection of EST clusters 1-3

As discussed above it is possible that each EST cluster represents a single gene, alternatively the EST clusters may be portions of the same gene. To distinguish between these two possiblities, primers were designed to the two other EST clusters in the region represented by EST accession numbers F07016 (cluster 2, containing 272 nucleotides) and R50627 (cluster 3, containing 1177 nucleotides). Primers from cluster 1 (117F and 499F) were paired with a primer from EST cluster 3 (4034R) in a PCR reaction. A 50 ul reaction was performed using the Takara LA Taq polymerase (Panvera, Madison, WI) in the reaction buffer supplied by the manufacturer with the addition of 0.32 mM dNTPs, primers, and approximately 30ng of lymph node cDNA. PCR products were amplified for 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 4 minutes. Products were electrophoresed on a 1% agarose gel and bands of 2.5 to 3 kb were excised, subcloned into pCR 2.1 (Invitrogen, Carlsbad, CA), and plasmid DNA was prepared for DNA sequence analysis.

The primary reaction described above generated by a primer in EST cluster 1 (638F) and EST cluster 3 (4173R) was utilized as the template for a reaction with a primer from EST cluster 1 (638F) and from EST cluster 2 (3556R). This semi-nested PCR reaction was performed with Takara LA Taq polymerase as described in the previous paragraph. An approximately 2 kb product was generated and subcloned for DNA sequence analysis. The assembly of the DNA sequence results of these PCR products indicated that EST clusters 1 to 3 were part of the same gene and established their orientation relative to each other in the mRNA transcript produced by this gene.

PCR reactions were also performed between EST clusters 2 and 3. Amplification from liver cDNA using Takara LA Taq polymerase (Panvera, Madison, WI) with the primers 2519F, 3011F, or 3154F (EST cluster 2) in combination with 5061R (EST cluster 3) was done for 35 cyles of 95°C for 30 sec, 60°C for 60 sec, and 72°C for 3 minutes. The PCR products were gel purified, subcloned, and the DNA sequence was determined. The DNA sequence analysis of the ends of all these PCR products resulted in most of the cDNA sequence however to provide for

complete DNA sequence of both strands oligonucleotide primers were designed and used for DNA sequencing (Figure 5(a)).

Extension of the 5' end

RCCA analysis was utilized to obtain a number of clones extended 5' by using the internal gene specific primers as described previously. Several clonal extensions were isolated however most of the clones analyzed stopped within exon A. One clone extended past the 5' end of exon A but the sequence was contiguous with genomic DNA, since a body of evidence indicates an intron/exon boundary at the 5' end of exon A it appeared likely that this extension is a result of unprocessed intronic sequence. A second clone h10 extended past this point but diverged from the genomic DNA sequence. It was concluded that this represented a chimeric clone that was present in the original fetal brain cDNA library.

Identification of 5' end of isoform 1

As described above results from RCCA experiments yielded a number of independent clones that terminated at the 5' end of exon A. This suggested that the human *LRP5* gene contains a region that the reverse transcriptase has difficulty transcribing. To circumvent this problem we decided to isolate the mouse ortholog of *LRP5*, since subtle differences in DNA sequence content can alter the ability of an enzyme to transcribe a region. To increase the probability of isolating the 5' portion of the mouse gene a human probe of 366 nucleotides, described above and derived from exons A and B was used.

A cDNA library was constructed from mouse liver mRNA purchased from Clontech (Palo Alto, CA). cDNA was prepared using the SuperScript Choice system (Gibco/BRL Gaithersburg, MD) according to the manufacturer's instructions. Phosphorylated *Bst* XI adapters (Invitrogen, San Diego, CA) were ligated to approximately 2 ug of mouse liver cDNA. The ligation mix was diluted and size-fractionated on a cDNA sizing column (Gibco/BRL Gaithersburg, MD). Drops from the column were collected and the eluted volume from the column determined as described for the construction of shotgun

libraries. The size-fractionated cDNA with the *Bst* XI linkers was ligated into the vector pSHOT II, described above, cut with the restriction endonuclease *Bst* XI, gel purified, and dephosphorylated with calf intestinal phosphatase (Gibco/BRL, Gaithersburg, MD). The ligation containing approximately 10-20 ng of cDNA and approximately 100 ng of vector was incubated overnight at 14°C. The ligation was transformed into XL-2 Blue Ultracompetent cells (Stratagene, La Jolla, CA.). The transformed cells were spread on twenty 133 mm Colony/Plaque Screen filters (Dupont/NEN, Boston, MA.) at a density of approximately 30,000 colonies per plate on Luria Broth agar plates supplemented with 100 ug/ml ampicillin (Sigma, St. Louis, MO.). The colonies were grown overnight and then replica plated onto two duplicate filters. The replica filters were grown for several hours at 37°C until the colonies were visible and processed for in situ hybridization of colonies according to established procedures (Maniatis, Fritsch and Sambrook, 1982). A Stratalinker (Stratagene, La Jolla, CA.) was used to crosslink the DNA to the filter. The filters were hybridized overnight with greater than 1,000,000 cpm/ml probe in 1X hybridization buffer (Gibco/BRL, Gaithersburg, MD) containing 50% formamide at 42°C. The probe was generated from a PCR product derived from the human *LRP5* cDNA using primers 512F and 878R. This probe was random prime labeled with the Amersham Rediprime kit (Arlington Heights, IL) in the presence of 50-100 uCi of 3000 Ci/mmole [alpha 32P]dCTP (Dupont/NEN, Boston, MA) and purified using a ProbeQuant G-50 spin column (Pharmacia/Biotech, Piscataway, NJ). The filters were washed with 0.1X SSC, 0.1% SDS at 42°C. Following autoradiography individual regions containing hybridization positive colonies were excised from the master filter and placed into 0.5 ml Luria Broth plus 20% glycerol. Each positive was replated at a density of approximate 50-200 colonies per 100 mm plate and screened by hybridization as described above. Single colonies were isolated and plasmid DNA was prepared for DNA sequence analysis.

Three clones were isolated from the mouse cDNA library the assembled sequence of the clones (Figure 16(a)) that had a high degree of similarity (87% identical over an approximately

1700 nucleotide portion) with the human *LRP5* gene and thus likely represent the mouse ortholog of *LRP5*. The 500 amino acid of the portion of the mouse LRP5 (Figure 16(d)) that we initially obtained is 96% identical to human LRP5. Significantly two of these clones had sequence that was 5' of the region corresponding to exon A, clone 19a contained an additional 200 bp and clone 9a contained an additional 180 bp (Figure 16(b)). The additional 200 bp contains an open reading frame that begins at bp 112 (Figure 16(c)). The initiating codon has consensus nucleotides for efficient initiation of translation at both the -3 (purine) and +4 (G nucleotide) positions (Kozak, M. 1996, Mamalian Genome 7:563-574). This open reading frame encodes a peptide with the potential to act as a eukaryotic signal sequence for protein export (von Heijne, 1994, Ann. Rev. Biophys. Biomol. Struc. 23:167-192). The highest score for the signal sequence as determined by using the SigCleave program in the GCG analysis package (Genetics Computer Group, Madison WI) generates a mature peptide beginning at residue 29 of isoform 1. Additional sites that may be utilized produce mature peptides beginning at amino acid residue 31 (the first amino acid encoded by exon A) or amino acid residues 32, 33, or 38.

Molecular cloning of the full length mouse Lrp3 cDNA

The mouse cDNA clones isolated by nucleic acid hybridization contain 1.7 Kb of the 5' end of the *Lrp3* cDNA (Figure 16(a)). This accounts for approximately one-third of the full length cDNA when compared to the human cDNA sequence. The remainder of the mouse *Lrp3* cDNA was isolated using PCR to amplify products from mouse liver cDNA. PCR primers, Table 9, were designed based upon DNA sequences identified by the sequence skimming of mouse genomic clones, BACs 53-d-8 and 131-p-15, which contain the mouse *Lrp3* gene. BAC 53-d-8 was mapped by FISH analysis to mouse chromosome 19 which is syntenic with 11q13. Sequence skimming of these clones identified DNA sequences that corresponded to the coding region of human *LRP5* as well as the 3' untranslated region. This strategy resulted in the determination of a mouse cDNA sequence of 5059 nucleotides (Figure 18(a)) which contains an

open reading frame of 4842 nucleotides (Figure 18(b)) that encodes a protein of 1614 amino acids (Figure 18(c)). The putative ATG is in a sequence context favorable for initiation of translation (Kozak, M. 1996, Mamalian Genome 7:563-574).

Comparison of human and mouse LRP5

The cDNA sequences of human and mouse *LRP5* display 87% identity. The open reading frame of the human *LRP5* cDNA encodes a protein of 1615 amino acids that is 94% identical to the 1614 amino acid protein encoded by mouse *Lrp3* (Figure 18(d)). The difference in length is due to a single amino acid deletion in the mouse Lrp3 signal peptide sequence. The signal peptide sequence is not highly conserved being less than 50% identical between human and mouse. The location of the putative signal sequence cleavage site is at amino acid residue 25 in the human and amino acid 29 in the mouse. Cleavage at these sites would result in mature human and mouse proteins of 1591 and 1586 amino acids, respectively, which are 95% identical (Figure 18(e)). The high degree of overall sequence similarity argues strongly that the identified sequences are orthologs of the *LRP5* gene. This hypothesis is further supported by the results of genomic Southern experiments (data not shown).

Identification of human signal peptide exon for isoform 1

The human exon encoding a signal peptide was isolated from liver cDNA by PCR. The forward primer 1F (Table 9) was used in combination with one of the following reverse primers: 218R, 265R, 318R, and 361R in a PCR reaction using Taq Gold polymerase (Perkin-Elmer, Norwalk, CT) and supplemented with either 3, 5, or 7% DMSO. Products were amplified for 40 cycles of 30 sec 95°C, 30 sec 58°C, and 1 min 72°C. The products were analyzed on an agarose gel and some of the reactions containing bands of the predicted size were selected for DNA sequence analysis and subcloning into pCR2.1 (Invitrogen, San Diego, CA).

The derived DNA sequence of 139 nucleotides upstream of exon 2 (also known as exon A) contains an ATG that is in a context for efficient initiation of translation: an adenine

(A) residue at the -3 position and a guanine (G) residue at the +4 position (Kozak, M. 1996, Mamalian Genome 7:563-574). The open reading frame for this ATG continues for 4854 nucleotides (Figure 5(b)) which encodes a polypeptide of 1615 amino acids (Figure 5(c)

The sequence following the initiator ATG codon encodes a peptide with the potential to act as a signal for protein export. The highest score for the signal sequence (15.3) indicated by the SigCleave program in the GCG analysis package (Genetics Computer Group, Madison WI) generates a mature polypeptide beginning at amino acid residue 25 (Figure 5(d,e). Additional putative cleavage sites that may be utilized to produce a mature LRP5 protein are predicted for residues 23, 24, 26, 27, 28, 30 and 32 (the first amino acid encoded by exon A).

Determination of the genomic DNA sequence containing and flanking the signal peptide exon

The region that contained genomic DNA sequence identical to the cDNA sequence encoding a signal peptide was in a gap between two stretches of contiguous genomic DNA sequence known as contigs 57 and 58. To close this gap four clones were chosen from the shotgun library that were determined to span this gap according to analysis by the program Phrapview licensed from Dr. Phil Green of the University of Washington (Seattle, WA). Direct DNA sequencing of these clones was unsuccessful, i.e. high GC content significantly reduced the efficiency of the cycle sequencing. To circumvent this problem PCR products were generated by incorporating 7-deaza-dGTP (Pharmacia, Pharmacia Biotech, Piscataway, NJ). The conditions for these reactions consisted of a modification of the Klentaq Advantage-GC polymerase kit (Clontech, Palo Alto, CA). The standard reaction protocol was modified by supplementing the reaction mix with 200 uM 7-deaza-dGTP. Inserts were amplified with M13 forward and reverse primers for 32 cycles of 30 sec at 92°C, 1 min at 60°C, and 5 min at 68°C. Products were gel purified using Qiaquick gel extraction kit (Qiagen Inc., Santa Clarita, CA) and sequenced as described previously. Assembly of the resulting sequences

closed the gap and generated a contiguous sequence of approximately 78,000 bp of genomic DNA.

Extension of Isoforms 2 and 3

The software package GRAIL (supra) predicts exons and promoter sequences from genomic DNA sequence. One region identified by GRAIL is an exon originally designated G1 and subsequently termed exon 1 that is approximately 55 kb upstream of the beginning of exon A (Figure 12(c)). Three primers designated G1 1f to 3f were designed based on this sequence. This exon was of particular interest because GRAIL also predicted a promoter immediately upstream of the exonic sequence (Figure 12 (e)). Furthermore one of the open reading frames in G1 encoded a peptide that had the characteristics of a eukaryotic signal sequence.

To determine whether the G1 predicted exon was part of the *LRP5* gene, reverse transcriptase (RT) PCR was performed using the Taqara RNA PCR kit (Panvera, Madison WI). Human liver mRNA (50 ng) was used as the template for a 10 ul reverse transcriptase reaction. The reverse transcriptase reaction using one of the *LRP5* specific primers (622R, 361R, or 318R) was incubated at 60°C for 30 min, followed by 99°C for 5 min, and then the sample was placed on ice. One of the forward primers, Table 2, (G1 1f, 2f, or 3f) was added along with the reagents for PCR amplification and the reaction was amplified for 30 cycles of 30 sec at 94°C, 30 sec at 60°C, and 2 min at 72°C. This primary PCR reaction was then diluted 1:2 in water and 1 ul of the reaction was used in a second 20 ul reaction using nested primers. The reaction conditions for the second round of amplification were 30 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 2 min. The products were separated on an agarose gel and excised. The purified fragments were subcloned into pCR 2.1 (Invitrogen, Carlsbad, CA), plasmid DNA was prepared, and the DNA sequence was determined.

The DNA sequence of these products indicated that G1 (exon 1) was present on at least a portion of the *LRP5* transcripts. Two different isoforms were identified. The first, isoform 2 (Figure 11(a)), identified in this experiment

consists of exon 1 followed by an exon that we have given the designation exon 5. This splice variant has an open reading frame that initiates in exon B nucleotide 402 (Figure 11 (a)), the initiator methionine at this location does not conform to the consensus sequences for translation initiation (Kozak, M. (1996) Mamalian Genome 7:563-574). A second potential initiator methionine is present at nucleotide 453, this codon is in a context for efficient initiation of translation initiation (Kozak, M. (1996) Mamalian Genome 7:563-574). The longest potential open reading frame for isoform 2 (Figure 11 (c)) encodes a splice variant contains a eukaryotic signal sequence at amino acid 153. The mature peptide generated by this splice variant would be lacking the first five spacer domains and a portion of the first EGF-like motif.

The second isoform (isoform 3) consists of exon 1 followed by exon A (Figure 12 (a)). It is not known whether exon 1 is the first exon of isoform 2. However the location of a GRAIL predicted promoter upstream of G1 suggests the possibility that exon 1 is the first exon. Futhermore there is an open reading frame that extends past the 5' intron/exon boundary postulated by GRAIL (Figure 12 (b)). Therefore we have examined the possiblity of incorporating this extended open reading frame into the *LRP5* transcript. The resulting open reading frame (Figure 12 (c)) encodes a 1639 amino acid protein (Figure 12 (d). The initiator methionine codon does not contain either of the consensus nucleotides that are thought to be important for efficient translation (Kozak, M. 1996, Mamalian Genome 7:563-574). Nor does the predicted protein contain a predicted eukaryotic signal sequence within the first 100 amino acids. Alternatively there may be additional exons upstream of exon 1 which provide the initiator methionine codon and/or a potential signal sequence.

RACE extension of the 5' end of lrp5: Isoforms 4 and 5

RACE is an established protocol for the analysis of cDNA ends. This procedure was performed using the Marathon RACE template purchased from Clontech (Palo Alto, CA). This was performed according to instructions using Clontech "Marathon" cDNA from fetal brain and mammary tissue. Two "nested" PCR

amplifications were performed using the ELONGASE™ long-PCR enzyme mix & buffer from Gibco-BRL (Gaithersburg, MD).

Marathon primers

AP1: CCATCCTAATACGACTCACTATAGGGC

AP2: ACTCACTATAGGGCTCGAGCGGC

First round PCR used 2 microliters Marathon placenta cDNA template and 10 pmoles each of primers L217 and AP1. Thermal cycling was: 94°C 30 sec, 68°C 6 min, 5 cycles; 94°C 30 sec, 64°C 30 sec, 68°C 4 min, 5 cycles; 94°C 30 sec, 62°C 30 sec, 68°C 4 min, 30 cycles. One microliter from a 1/20 dilution of this reaction was added to a second PCR reaction as DNA template. This PCR reaction also differed from the first PCR reaction in that nested primers L120 and AP2 were used. Two products of approximately 1600 bp and 300 bp were observed and cloned into pCR2.1 (Invitrogen, Carlsbad CA). The DNA sequence of these clones indicated that they were generated by splicing of sequences to exon A. The larger 1.6 kb fragment (Figure 13) identified a region approximately 4365 nucleotides upstream of exon A and appeared to be contiguous with genomic DNA for 1555 base pairs. The sequence identified by the 300 bp fragment was approximately 5648 nucleotides upstream of exon A (Figure 14). This sequence had similarity to Alu repeats. The region identified by the 300 bp fragment was internal to the region identified by the 1.6 kb fragment. The open reading frame for these isoforms designated 4 and 5 is the same as described for isoform 2 (Figure 11 (b)).

Extension of Isoform 6

GRAIL (supra) analysis was used to predict potential promoter regions for the gene. Primers were designed to the isoform 6 promoter sequence (Figure 15 (b)) which was defined by GRAIL and is approximately 4 kb centromeric of exon A. This region was designated GRAIL promoter-1 (Gp-1).

The PCR primer Gp 1f (Table 2) was used in a PCR reaction with primer 574r and 599r using the polymerase Taq Gold in the reaction buffer supplied by the manufacturer (Perkin Elmer, Norwalk, CT). The reaction conditions were 12 min at 95°C followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and

72°C for 1 min 30 sec with approximately 10ng of liver cDNA per 20 ul reaction. The primary reactions were diluted 20 fold in water and a second round of PCR using primer Gp 1f in combination with either 474r or 521r was done. Products were analyzed on a 2% agarose gel and bands of approximately 220 to 400 bp were subcloned into pCR 2.1 (Invitrogen, Carlsbad, CA) and analyzed by DNA sequence analysis. The open reading frame present in isoform 4 is the same as described for isoform 2 above (Figure 11 (b)).

Microsatellite Rescue

A vectorette library was made from each clone by restricting each clone and ligating on a specific bubble linker (Munroe, D.J. et al. (1994) Genomics 19, 506). PCR was carried out beween a primer (Not 1-A) specific for the linker, and a repeat motif (AC)11N, (where N is not A), at an annealing temperature of 65°C. The PCR products were gel purified and sequenced using the ABI PRISM dye terminator cycle sequencing kit as previously described. From this sequence, a primer was designed, which was used in PCR with the Not 1-A primer. This was also sequenced, and a second PCR primer designed, (Table 8) so that both primers flanked the repeat motif, and were used for genotyping.

Mutation Scanning

Single nucleotide polymorphisms (SNP's) were identified in type 1 diabetic patients using a sequencing scanning approach (Table 5).

Primers were designed to specifically amplify genomic fragments, approximately 500 to 800 bp in length, containing specific regions of interest (i.e. regions that contained *LRP5* exons, previously identified SNP's or GRAIL predicted exons). To facilitate fluorescent dye primer sequencing, forward and reverse primer pairs were tailed with sequences that correspond to the M13 Universal primer (5'-TGTAAAACGACGGCCAGT-3') and a modified M13 reverse primer (5'-GCTATGACCATGATTACGCC-3'), respectively. PCR products produced using the primer sets, mentioned above, were amplified in 50 ul reactions consisting of Perkin-Elmer 10 x PCR Buffer, 200

mM dNTP's, 0.5 ul of Taq Gold (Perkin-Elmer Corp., Foster City, CA), 50 ng of patient DNA and 20 pmol/ml of forward and reverse primers. Cycling conditions were 95°C for 12 min; 35 cycles of 95°C for 30 sec, 57°C for 30 sec and 68°C for 2 min, followed by an extension of 72°C for 6 min and a 4°C hold.

Conditions were optimized so that only single DNA fragments were produced by these reaction. The PCR products were then purified for sequencing using QiaQuick strips or QiaQuick 96 well plates on the Qiagen robot (Qiagen Inc., Santa Clarita, CA). This purification step removes the unincorporated primers and nucleotides.

Direct BODIPY dye primer cycle sequencing was the method used to analyze the PCR products (Metzker *et. al.* (1996) Science 271, 1420-1422). A Tecan robot (Tecan, Research Triangle Park, NC) carried out the sequencing reactions using standard dye primer sequencing protocols (ABI Dye Primer Cycle Sequencing with AmpliTaq DNA Polymerase FS, Perkin-Elmer Corp., Foster City, CA). The reactions were generated using the following cycling conditions on a DNA Engine thermal cycler (M.J. Research Inc., Watertown, MA), 15 cycles of 95°C for 4 sec, 55°C for 10 sec, and 70°C for 60 sec; followed by 15 cycles of 95°C for 4 sec, and 70°C for 60 sec. After cycling, samples were pooled, precipitated and dried down. The samples were resuspended in 3 ul of loading buffer and 2 ml were run on an ABI 377 Automated DNA sequencer.

Once SNP's have been identified, scanning technologies are employed to evaluate their informativeness as markers to assist in the determination of association of the gene with disease in the type 1 diabetic families. We are using restriction fragment length polymorphisms (RFLP's) to assess SNP's that change a restriction endonuclease site. Furthermore, we are using forced RFLP PCR (Li and Hood (1995) Genomics 26, 199-206; Haliassos *et.al.* (1989) Nuc. Acids Res. 17, 3608) and ARMS (Gibbs *et.al.* (1989) Nuc. Acids Res. 17, 2437-2448; Wu *et. al.* (1989) Proc. Natl. Acad. Sci. USA 86, 2757-2760) to evaluate SNP's that do not change a restriction endonuclease site. We are also trying to scan larger regions of the locus by developing fluorescent based Cleavase (CFLP)

(Life Technologies, Gaithersburg, MD) and Resolvase, (Avitech Diagnostics, Malvern, PA) assays.

Haplotype analysis at IDDM4

Haplotype mapping (or identity-by-descent mapping) has been used in conjunction with association mapping to identify regions of identity-by-descent (IBD) in founder populations, where (some) of the affected individuals in a founder population share not only the mutation, but also a quite large genomic haplotype (hence identical piece of DNA) surrounding the disease locus. Recombinant haplotypes can be utilised to delineate the region containing the mutation. These methods have been used to map the genes of the recessive disorders: Wilson's disease, Batten's disease, Hirschsprung's disease and hereditary haemochromatosis (Tanzi, R., *et al.* (1993) *Nature Genet* 5, 344-350; The International Batten Disease Consortium. (1995) *Cell* 82, 949-957; Puffenberger, E., *et al.* (1994) *Hum Mol Genet* 3, 1217-1225; and Feder, J., *et al.* (1996) *Nature Genet* 13, 399-408). Similarly, in type 1 diabetes, for *IDDM1*, comparative MHC haplotype mapping between specific Caucasian and haplotypes of African origin identified both *HLA-DQA1* and *HLA-DQB1* as susceptibility loci for this disorder (Todd, J. *et al* (1989) *Nature* 338, 587-589; and Todd, J. *et al* (1987) *Nature* 329, 599-604).

On chromosome 11q13 haplotype analysis was undertaken in conjunction with association analysis in order to identify regions of IBD between haplotypes which are transmitted more often than expected, hence contain a susceptible allele at the aetiological locus; in contrast protective haplotypes will be transmitted less often than expected and contain a different (protective) allele at the aetiological locus. Evidence for a deviation in the expected transmission of alleles was shown with the two polymorphic markers *D11S1917* and *H0570POLYA* . In 2042 type 1 diabetic families from the UK, USA, Norway, Sardinia, Romania, Finland, Italy and Denmark, transmission of *D11S1917-H0570POLYA* haplotype 3-2 to affected offspring was negative (46%), with a 2X2 test of heterogeneity between affected and unaffected transmissions produced $\chi^2=23$, df=1, $p<1.5 \times 10^{-6}$, providing good evidence that this is a

protective haplotype. In contrast, the 2-3 haplotype was more transmitted to affected than non-affected offspring (%T=51.3; 2X2 contingency test; χ^2=5.5, df=1, p<0.02), indicating that this was a susceptible (or possibly neutral) chromosome. A further haplotype, which is rare, has been identified which appears to be susceptible to type 1 diabetes (*D11S1917-H0570POLYA*, 3-3, %T affecteds = 62.4, 2X2 contingency test, affecteds *vs* non-affecteds;chi^2=6.7, df=1, p<0.009). Therefore, analysis of association in this region has produced evidence for a haplotype which contains an allele protective against type 1 diabetes, as it is significantly less transmitted to the affected offspring in comparison to the unaffected offspring, and evidence for two non-protective haplotypes, which have a neutral or susceptible effect on type 1 diabetes.

Extending this haplotype analysis to include the 14 flanking microsatellite markers *255ca5, D11S987, 255ca6, 255ca3, D11S1296, E0864CA, TAA, L3001CA, D11S1337, 14LCA5, D11S4178, D11S970, 14LCA1, 18O18*, as well as the single nucleotide polymorphisms (SNPs) *58-1, Exon E* (intronic, 8bp 3' of exon 6) and *Exon R* (Ala ^{1330}, exon 18) (Figure 19), revealed highly conserved haplotypes within this interval in the diabetic individuals. A distinct protective haplotype (A) has been identified (encompassing the 3-2 haplotype at *D11S1917-H0570POLYA*), as well as a distinct susceptible haplotype (B) (encompassing the 2-3 haplotype at *D11S1917-H0570POLYA*). The susceptible haplotype is IBD with the protective haplotype, 3' of marker *D11S1337*, indicating that the aetiological variant playing a role in type 1 diabetes does not lie within the identical region, localising it 5' of *Exon E* of the *LRP-5* gene. This region that is IBD between the protective, and susceptible haplotypes prevents association analysis being undertaken, as no deviation in transmission to affected offspring would be detected. The rare susceptible haplotype (C), 3-3 at *D11S1917-H0570POLYA*, can also be identified. Haplotype analysis with the additional markers in the region reveals that this rare susceptible haplotype is identical to the susceptible haplotype between *UT5620* and *14L15CA*, potentially localising the aetiological variant between *UT5620*

and *Exon E*, which is approximately 100kb. Therefore, the susceptible and rare susceptible haplotypes may carry an allele (or separate alleles) which confers a susceptible effect on type 1 diabetes, whereas the protective haplotype contains an allele protective against *IDDM*. The 5' region of the *LRP5* gene lies within this interval, encompassing the 5' regulatory regions of the *LRP5* gene and exons 1 to 6.

Analysis of the Italian and Sardinian haplotypes revealed an additional two susceptible haplotypes. At *D11S1917-H0570POLYA* in the Italian families haplotype 1-3, 63%T, 2X2 affected verses non-affecteds p=0.03 (haplotype D). At *H0570POLYA -L3001* in the Sardinian families haplotype 1-2 58%T, 2X2 affected verses non-affecteds, p=0.05 (haplotype E).

Samples containing the above five haplotypes were genotyped with SNPs from the *IDDM4* region in order to investigate regions of IBD (Figure B). These SNPs confirmed the region of IBD between the susceptible haplotypes B and C between *UT5620* and *14L15CA*. It also confirmed the region of IBD between the protective and susceptible haplotypes A and B 3' of marker *D11S1337*, excluding this region from containing the aetiological variant. The SNP analysis also revealed a potential region of IBD between *UT5620* and *TAA*, between the susceptible haplotypes B, C, D and E, which is distinct from the protective haplotype A (a 25kb region). The marker *H0570POLYA* lies within this interval, and is not identical in haplotype E compared to the other susceptible haplotypes; possibly this is due to mutation at this polymorphism, or it delineates a boundary within this region and the aetiological variant is either 5' or 3' of this marker. Further analysis of additional SNPs within this interval will be necessary.

Therefore haplotype mapping within the *IDDM4* region has identified a region of IBD between the susceptible haplotypes B and C of 100kb, in the 5' region of the LRP5 gene. SNP haplotype mapping has possibly further delineated this to a 25kb interval encompassing the 5' region of LRP5 which includes possible regulatory sequences for this gene; a putative promoter, and regions of homology with the mouse syntenic region (Table 12), as well as exon 1 of LRP5.

Construction of Adenovirus vectors containing LRP5

The full-length human *LRP5* gene was cloned into the adenovirus transfer vector pdelE1sp1A-CMV-bGHPA containing the human Cytomegalovirus immediate early promoter and the bovine growth hormone polyadenylation signal to create pdehlrp3. This vector was used to construct an adenovirus containing the *LRP5* gene inserted into the E1 region of the virus directed towards the 5' ITR. In order to accommodate a cDNA of this length, the E3 region has been completely deleted from the virus as it has been described for pBHG10 (Bett at al.1994 Proc Natl Acad Sci 91: 8802-8806) An identical strategy was used to construct an adenoviral vector containing the full-length mouse *Lrp5* gene.

A soluble version of mouse *Lrp5* was constructed in which a His tag and a translational stop signal replaced the putative transmembrane spanning domain (primers listed in Table 9). This should result in the secretion of the extracellular domain of Lrp5 and facilitate the biochemical characterization of the putative ligand binding domain of Lrp5. Similarly a soluble version of human LRP5 can be constructed using primers shown in Table 9. The extracellular domain runs to amino acid 1385 of the precursor (immature) protein sequence.

Identification of LRP5 ligands

LRP5 demonstrates the ablility to bind and take up LDL (see below), but this activity is not a high level. Therefore, it is likely that LRP5 has the capacity to bind additional ligand(s). To identify LRP5 ligands the extracellular domain consisting of the first 1399 amino acids of human LRP5, or the corresponding region of mouse Lrp5 will be purified. A number of expression systems can be used these include plasmid based systems in *Drosophila* S2 cells, yeast and *E. coli* and viral based systems in mammalian cells and SF9 insect cells. A histidine tag will be used to purify LRP5 on a nickel column (Novagen, Madison WI). A variety of resins may be used in column chromatography to further enrich soluble LRP5. LRP5 will be attached to a solid support e.g. a nickel

column. Solutions containing ligands from serum fractions, urine fractions, or fractions from tissue extracts will be fractionated over the LRP5 column. LRP5 complexed with bound ligand will be eluted from the nickel column with imidizole. The nature of the ligand(s) bound to LRP5 will be characterized by gel electrophoresis, amino acid sequence, amino acid composition, gas chromatography, and mass spectrophotometer.

Attachment of purified LRP5 to a BiaCore 2000 (BiaCore, Uppsula Sweden) chip will be used to determine whether ligands that bind to LRP5 are present in test solutions. Once ligands for LRP5 are identified the LRP5 chip will be used to characterize the kinetics of the LRP5 ligand interaction.

Adenoviral vectors containing soluble versions of LRP5 will be used to infect animals, isolation of ligand/LRP5 complexes from serum or liver extracts will be facilitated by the use of a histidine tag and antibodies directed against this portion of LRP5.

Treatment of animals with LRP5 virus

A wide range of species may be treated with adenovirus vectors carrying a transgene. Mice are the preferred species for performing experiments due to the availability of a number of genetically altered strains of mice, i.e. knockout, transgenic and inbred mice. However larger animals e.g. rats or rabbits may be used when appropriate. A preferred animal model to test the ability of *LRP5* to modify the development of type 1 diabetes is the non-obese diabetic (NOD) mouse. Preferred animal models for examination of a potential role for *LRP5* in lipoprotein metabolism are mice in which members of the LDL-receptor family have been disrupted, e.g. the LDL-receptor (*LDLR*), or in which genes involved in lipoprotein metabolism, e.g. *Apo-E*, have been disrupted.

Adenoviruses are administered by injecting approximately 1×10^9 plaque forming units into the tail vein of a mouse. Based on previous studies this form of treatment results in the infection of hepatocytes at a relatively high frequency. Three different adenovirus treatments were prepared, 1.) adenovirus containing no insert (negative control), 2.)

adenovirus containing human *LDLR* (positive control) or 3.) adenovirus containing human *LRP5*. Each of these viruses were used to infect five C57 wild type and five C57 LDLR knockout mice. A pretreatment bleed, 8 days prior to injection of the virus was used to examine serum chemistry values prior to treatment. The animals were injected with virus. On day five following administration of the virus a second (treatment) bleed was taken and the animals were euthanized for collection of serum for lipoprotein fractionation. In addition tissues were harvested for in situ analysis, immunohistochemistry, and histopathology.

Throughout the experiment, animals were maintained in a standard light/dark cycle and given a regular chow diet. The animals were fasted prior to serum collection. In certain experimental conditions it may be desirable to give animals a high fat diet.

Standard clinical serum chemistry assays were performed to determine; serum triglycerides, total cholesterol, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, urea nitrogen, and creatinine. Hematology was performed to examine the levels of circulating leukocytes, neutrophils, the percent lymphocytes, monocytes, and eosinophils, erythrocytes, platelets, hemoglobin, and percent hematocrit.

Serum lipoproteins were fractionated into size classes using a Superose 6 FPLC sizing column and minor modifications of the procedure described by Gerdes et al. (Clin. Chim. Acta 205:1-9 (1992)), the most significant difference from the Gerdes procedure being that only one column was used. Column fractions were collected and analyzed for cholesterol and triglyceride. The "area under the curve" was calculated for each lipoprotein class. The approximate peak fractions that correspond to each of the classes defined by density are: fraction 24 for VLDL, fraction 36 for LDL and fraction 51 for HDL.

LRP5 overexpression affects serum triglycerides and lipoproteins

Statistical analysis of serum chemistry data indicated

that relative to control virus there was a 30% decrease, p value = 0.025, in triglyceride levels in animals treated with *LRP5* containing virus (Table 10). This decrease in triglycerides occurred at a similar level in both wild type and KO mice. By comparison, the *LDLR* virus reduced serum triglycerides approximately 55%, relative to the contol virus. This result indicates that *LRP5* has the potential to modulate serum triglyceride levels.

The serum lipoprotein profile indicated that the VLDL particle class was decreased in wild type mice treated with *LRP5* virus. Although the number of samples analyzed was not sufficient for statistical analyses, this result is consistent with the observed decrease in serum triglycerides. These results suggest that LRP5 has the potential to bind and internalize lipid rich particles, causing the decrease in serum triglycerides and VLDL particles. Therefore treatment with *LRP5* or with therapeutic agents that increase the expression of *LRP5* or the biological activity of LRP5 may be useful in reducing lipid rich particles and triglycerides in patients with diseases that increase triglyceride levels, e.g. type 2 diabetes and obesity.

Although not statistically significant there was an observed trend towards a reduction in serum cholesterol levels as a consequence of LRP5 treatment (28 %, p = 0.073) in mice that have a high level of serum cholesterol (approximately 220 mg/dL), due to a disruption (knockout) of the LDL-receptor (Table 10). An opposite trend, in that LRP5 treatment elevated serum cholesterol (30%, p = 0.08) was not observed in wild type mice which have a relatively low level of serum cholesterol (approximately 70 mg/dL). The small treatment groups, n = 4 , in these data sets limits the interpretation of these results and indicates that further experimentation is necessary. Nevertheless, these results suggest that in a state of elevated cholesterol an increase in the activity of LRP5 might reduce serum cholesterol levels. Therefore treatment with *LRP5* or with therapeutic agents that increase either the expression of *LRP5* or the biological activity of LRP5 may be useful in reducing cholesterol in patients with hypercholesterolemia.

LRP5 overexpression may affect serum alkaline phosphatase levels

Serum alkaline phosphatase levels can be dramatically elevated, e.g. 20 fold increase, as a consequence of an obstruction of the bile duct (Jaffe, M. S. and McVan, B., 1997, Davis's laboratory and diagnostic test handbook. pub. F.A. Davis Philadelphia PA). However, lower levels, up to a three fold increase of alkaline phosphatase can result from the inflammatory response that take place in response to an infectious agent in the liver, e.g. adenovirus. In animals treated with a control virus there was an approximately 2-fold increase in alkaline phosphatase levels. In contrast, there was only a slight increase in alkaline phophatase levels in animals treated with the *LRP5* virus. Relative to the control the alkaline phosphatase level was reduced 49% in the *LRP5* treated animals, p value = 0.001 (Table 10).

The increase in alkaline phosphatase levels may be a consequence of the level of infection with the adenovirus, therefore, a possible explanation for the decrease in the animals treated with the *LRP5* virus may simply be due to less virus in this treatment group. An indicator of the level of the viral infection is the appearance in the serum of the liver enzymes aspartate aminotransferase and alanine aminotransferase. These enzymes are normally found in the cytoplasm of cells and elevated in the serum when cellular damage occurs (Jaffe, M. S. and McVan, B., 1997, Davis's laboratory and diagnostic test handbook. pub. F.A. Davis Philadelphia PA). Therefore these enzymes serve as markers for the level of toxicity that is a consequence of the adenoviral infection. These enzymes are present at a normally low level prior to the infection and in animals that did not receive virus. Importantly, the levels of aspartate aminotransferase and alanine aminotransferase are higher in the animals given the *LRP5* virus indicating that these animals have more cellular damage and thus a more extensive infection than the animals given the control virus (Table 11). Therefore, it is unlikely that the reduced level of alkaline phosphatase is simply owing to less *LRP5* virus being administered. A second possible explanation is that LRP5 modifies the nature of the

inflammatory response resulting from the adenovirus infection. A possible role for *LRP5* in modulating the inflammatory response is consistent with the genetic data indicating that this gene is associated with risk for developing type 1 diabetes. Chronic insulitis or inflammation is a precursor to clinical onset of type 1 diabetes therefore *LRP5* treatment or treatment with therapeutic agents that either increase the transcription of *LRP5* may be of utility in preventing type 1 diabetes. Type 1 diabetes is an autoimmune disease, therefore treatment with *LRP5* or with therapeutics agents that either increase the expression of *LRP5* or the biological activity of LRP5 may be useful in treating other autoimmune diseases.

Expression of LRP5 in cell lines

Overexpression of *LRP5* under the control of a heterologous promoter can be accomplished either by infection with an adenovirus containing *LRP5* or by transfection with a plasmid vector containing *LRP5*. Transfection with a plasmid vector can lead to either transient or a stable expression of the transgene.

Endogenous LDL-receptors reduce the ability to detect the uptake of LDL by other members of the LDL-receptor family. To study lipoprotein uptake in the absence of the LDL-receptor, primary cell lines from human patients with familial hypercholesterolemia (FH) were used. These FH cell lines lack any endogenous LDL-receptor. FH fibroblasts were infected at an MOI of 500 plaque forming units per cell for 24 hours at 37°C. Following infection, cells were incubated with 40 μg/ml ^{125}I-LDL at 37°C. After 4 hours, cells were washed and uptake of LDL measured. A modest (approximately 60%) increase in the level of LDL uptake was observed. By comparison, the infection of FH cells with an adenovirus containing the LDL-receptor resulted in a 20-fold increase in LDL uptake ($p < 0.0001$, $n = 3$). To determine whether this modest level of activity mediated by LRP5 was statistically significant, 24 individual wells were infected with *LRP5* virus and analyzed. Statistical analysis of this experiment indicated that the increase in LDL uptake was highly signficant, $p < 0.0001$.

Therefore LRP5 can mediate LDL uptake. However, based on the modest level of activity, relative to the LDL-receptor, it does not appear that the primary activity of LRP5 is to mediate the uptake of LDL.

Additional cell lines exist that lack either the LDL-receptor or other members of the LDL-receptor family. The PEA-13 cell line (ATCC 2216-CRL) lacks the LRP1 receptor. Mutant CHO cells lacking the LDL receptor have been described by Kingsley and Krieger (Proceedings National Academy Sciences USA (1984) 81:5454). This cell line, known as *ldlA7*, is particularly useful for the creation of stable transfectant cell lines expressing recombinant LRP5.

Anti-LRP5 Antibodies

Western Blot Analysis

Antisera prepared in rabbits immunized with the human LRP5 MAP peptides

SYFHLFPPPPSPCTDSS

VDGRQNIKRAKDDGT

EVLFTTGLIRPVALVVDN

IQGHLDFVMDILVFHS

were evaluated by Western blot analysis.

COS cells were infected with an adenovirus containing human *LRP5* cDNA. Three days after the infection the cells were harvested by scraping into phosphate buffered saline (Gibco/BRL Gaithersburg, MD) containing the protease inhibitors PMSF (100ug/ml), aprotinin (2 ug /ml), and pepstatin A (1 ug/ml). The cells were pelleted by a low speed spin, resuspended in phosphate buffered saline containing protease inhibitors and lysed by Dounce homogenization. Nuclei were removed with a low speed spin, 1000 rpm for 5 min in a Beckman J-9 rotor. The supernatant was collected and centrifuged at high speed, 100,000 x g for 3 hours, to pellet the membranes. Membranes were resuspended in SDS-sample buffer (Novex, San Diego CA).

Membrane proteins were fractionated by electrophoresis on a 10% Tris-glycine acrylamide gel (Novex, San Diego CA). The fractionated proteins were transferred to PVDF paper (Novex, San Diego CA) according to the manufacturer's instructions.

Standard Western blot analysis was performed on the membrane with the primary antibody being a 1:200 dilution of crude antisera and the secondary antibody a 1:3000 dilution of antirabbit IgG HRP conjugate (Amersham, Arlington Heights, IL). ECL reagents (Amersham, Arlington Heights, IL) were used to visualize proteins recognized by the antibodies present in the sera.

A band of approximately 170-180 kD was detected by sera from a rabbit immunized with the peptide SYFHLFPPPPSPCTDSS. This band was only detected in the cells that were infected with the adenovirus containing human *LRP5* and was not present in cells that were infected with a control virus. Furthermore, the detection of this 170 kD band was blocked by preadsorbing a 1:500 dilution of the sera with 0.1 ug/ml of the peptide SYFHLFPPPPSPCTDSS but not with 0.1 ug/ml of the peptide VDGRQNIKRAKDDGT. Therefore this protein band of approximately 170 kD detected by the antibody directed against the peptide SYFHLFPPPPSPCTDSS is human LRP5. The predicted size of the mature human LRP5 protein is 176 kD.

The antisera from a rabbit immunized with the peptide SYFHLFPPPPSPCTDSS was affinity purified with an Affigel 10 column (BioRad, Hercules CA) to which the MAP peptide SYFHLFPPPPSPCTDSS was covalently attatched. This results in antisera with greater specificity for LRP5.

The antisera from a rabbit immunized with the peptide IQGHLDFVMDILVFHS is able to detect a band of approximately 170 kD that is present in cells infected with an LRP5 containing virus but not cells infected with a control virus. This antibody recognizes a peptide that is present in the putative extracellular domain of LRP5 and thus will be useful in detecting the soluble version of LRP5. However, there is greater background observed when using this antisera relative to that from the rabbit immunized with the peptide SYFHLFPPPPSPCTDSS.

LRP5 is expressed in tissue macrophages

The crude and affinity purified antisera to the LRP5 peptide SYFHLFPPPPSPCTDSS was used for immunocytochemistry studies in human liver. The antibody recognized tissue

macrophages, termed Kupfer cells in the liver, that stained positive for LRP5 and positive for the marker RFD7 (Harlan Bioproducts, Indianapolis IN) which recognizes mature tissue phagocytes and negative for an MHC class II marker, RFD1 (Harlan Bioproducts, Indianapolis IN). This pattern of staining (RFD1 - RFD7+) identifies a subpopulation of macrophages, the effector phagocytes. This class of macrophages has been implicated in the progression of disease in a model for autoimmune disease, experimental autoimmune neuritis (Jung. S. et al., 1993, J Neurol Sci 119: 195-202). The expression in phagocytic tissue macrophages supports a role for LRP3 in modulating the inflammatory component of the immune response. This result is consistent with the proposed role based on the differences observed in alkaline phoshatase levels in animals treated with LRP5 virus and the genetic data indicating that *LRP5* is a diabetes risk gene.

Determination of additional conserved regions of the LRP5 gene

High throughput DNA sequencing of shotgun libraries prepared from mouse BAC clones 131-p-15 and 53-d-8 was used to identify regions of the *LRP5* gene that are conserved between mouse and man. To identify these regions the mouse genomic DNA, either unassembled sequences or assembled contigs, was compared against an assembly of human genomic DNA. The comparison was done by using the BLAST algorithm with a cutoff of 80%. This analysis resulted in the identification of a majority of the exons of the *LRP5* gene and identified a number of patches of conserved sequences at other locations in the gene (Table 12).

There are sequences conserved between human and mouse located 4.3 kb and 168 bp upstream of the putative ATG. These sequences may represent 5′ untranslated sequences of the mRNA transcript or promoter elements.

Within the putative first intron of 36 kb there are twelve patches that exhibit a degree of DNA sequence conservation. Some of these regions, e.g. 41707-41903, are quite extensive and have a high degreee of sequence conservation, similar to that observed for the exons of the *LRP5* gene. Since these regions do not appear to be

transcribed it is likely that these conserved regions play a role in regulating either the transcription of the *LRP5* gene or the processing of the *LRP5* mRNA transcript. Regardless of exact nature of their role these newly identified regions represent areas where sequence polymorphism may affect the biological activity of LRP5.

The BAC clone 131-p-15 which contains the first two exons of *LRP5* was sequenced extensively, i.e. approximately 6X coverage. BAC clone 53-d-8 contains sequences from exon D to exon V, however the level of sequence coverage of this clone was only approximately 1X (skim sequencing). The skim sequencing of mouse BAC 53-d-8 resulted in 76% of the exons being detected, however in some instances only a portion of an exon was present in the mouse sequence data. In addition to the exons, there were three patches in the BAC 53-d-8 sequences that exhibited a degree of sequence conservation with the human sequences (Table 12). All of these were located in the large 20 kb intron between exons D and E. These sequences may represent regions that are important for the processing of this large intron and thus polymorphisms in these sequences may affect the expression level of *LRP5*.

Determination of relative abundance of alternatively spliced LRP5 mRNA transcripts

Several techniques may be used to determine the relative abundance of the different alternatively spliced isoforms of LRP5.

Northern blot analysis of probes derived from specific transcripts is used to survey tissues for the abundance of a particular transcript. More sensitive techniques such as RNase protection assays will be performed. Reagents from commercially available kits (Ambion, Inc. Austin TX) are used to prepare probes. The relative abundance of transcript that hybridizes to a probe radiolabeled with [alpha]32P-UTP is analyzed by native and denaturing acrylamide gels (Novex Inc., San Diego, CA). Primer extension assays are performed according to established procedures (Sambrook *et. al.* (1989) Molecular Cloning, Cold Spring Harbour Press, NY) using reverse primers derived from the 5' portion of the transcript.

Isolation of other species homologs of LRP5 gene

The *LRP5* gene from different species, e.g. rat, dog, are isolated by screening of a cDNA library with portions of the gene that have been obtained from cDNA of the species of interest using PCR primers designed from the human *LRP5* sequence. Degenerate PCR is performed by designing primers of 17-20 nucleotides with 32-128 fold degeneracy by selecting regions that code for amino acids that have low codon degeneracy e.g. Met and Trp. When selecting these primers preference is given to regions that are conserved in the protein e.g. the motifs shown in Figure 6b. PCR products are analyzed by DNA sequence analysis to confirm their similarity to human *LRP5*. The correct product is used to screen cDNA libraries by colony or plaque hybridization at high stringency. Alternatively probes derived directly from the human *LRP5* gene are utilized to isolate the cDNA sequence of *LRP5* from different species by hybridization at reduced stringency. A cDNA library is generated as described above.

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TABLE 1

Haplotype analysis at D11S1917(UT5620) - H0570POLYA, within 2582 families from UK, USA, Norway and Sardinia. Susceptible, protective and neutral alleles were identified at each polymorphism, and transmission of recombinant haplotypes to diabetic offspring was calculated (t=transmission, nt=non transmission). Significant transmission of the haplotype 332-104 was detected (P=0.005), as well as significant non-transmission of the haplotype 328-103 (P=0.03).

	D11S1917(UT5620)	*H0570POLYA*	t	nt	P
	328	104	539	474	
Protective	332	103	427	521	0.002
Susceptible	332	104	60	33	0.005
Protective	328	103	16	31	0.03

TABLE 2 PCR Primers for obtaining LRP5 cDNA

Primers located within *LRP5* cDNA:
The primers are numbered beginning at nucleotide 1 in Figure 5(a).

1F (muex 1f): ATGGAGCCCGAGTGAGC

218R (27R): ATGGTGGACTCCAGCTTGAC

256F (1F): TTCCAGTTTTCCAAGGGAG

265R (26R): AAAACTGGAAGTCCACTGCG

318R (4R): GGTCTGCTTGATGGCCTC

343F (2F): GTGCAGAACGTGGTCATCT

Vector Primers for RCCA

361R (21R): GTGCAGAACGTGGTCATCT

622R (2R): AGTCCACAATGATCTTCCGG

638F (4F): CCAATGGACTGACCATCGAC

657R (1R): GTCGATGGTCAGTCCATTGG

956R (22R): TTGTCCTCCTCACAGCGAG

1713F (21F): GGACTTCATCTACTGGACTG

1481R (23R): CAGTCTGTCCAGTACATGAG

1981F (22F): GCCTTCTTGGTCTTCACCAG

2261F (23F):GGACCAACAGAATCGAAGTG

2484R (5R): GTCAATGGTGAGGTCGT

2519F (5F): ACACCAACATGATCGAGTCG

3011F (24F): ACAAGTTCATCTACTGGGTG

3154F (25F): CGGACACTGTTCTGGACGTG

3173R (25R): CACGTCCAGAACAGTGTCCG

3556R (3R): TCCAGTAGAGATGCTTGCCA

Vector Primers for RCCA

3577F (3F): ATCGAGCGTGTGGAGAAGAC

4094F (30F): TCCTCATCAAACAGCAGTGC

4173R (6R): CGGCTTGGTGATTTCACAC

4687F (6F): GTGTGTGACAGCGACTACAGC

4707R (30R): GCTGTAGTCGCTGTCACACAC

5061R (7R): GTACAAAGTTCTCCCAGCCC

PCR primers in Sequences identified by GRAIL

G1 1F: TCTTCTCCAGAGGATGCAGC

G1 2F: TTCGTCTTGAACTTCCCAGC

G1 3F: TCTTCTTCTCCAGAGGATGCA

Gp1 1F: AGGCTGGTCTCAAACTCCTG

PBS.543R: GGGGATGTGCTGCAAGGCGA

PBS.578R: CCAGGGTTTTCCCAGTCACGAC

PBS.838F: TTGTGTGGAATTGTGAGCGGATAAC

PBS.873F: CCCAGGCTTTACACTTTATGCTTCC

Table 3 **Intron-Exon Organization of Human LRP5**

3' Acceptor Sequence Intron	Exon	Exon Number	Exon Size (bp)	5' Donor Sequence Exon	Intron	Intron Number & Size (bp)
ccgggtcaac/	ATGGAG	Ex 1 (6)	(91)	CCGCGG/	gtaggtgggc	1 (35051)
tgccccacag/	CCTCGC	Ex 2 (A)	(391)	TCACGG/	gtaaaccctg	2 (9408)
cccgtcacag/	GTACAT	Ex 3 (B)	(198)	GTTCCG/	gtaggtaccc	3 (6980)
ctgactgcag/	GCAGAA	Ex 4 (C)	(197)	CTTTCT/	gtgagtgccg	4 (1640)
gttttcccag/	TCCACA	Ex 5 (D)	(132)	AGGCAG/	gtgaggcggt	5 (20823)
gtctccacag/	GAGCCG	Ex 6 (E)	(397)	GATGGG/	gtaagacggg	6 (3213)
tcttctccag/	CCTCAT	Ex 7 (F)	(172)	ATCGAG/	gtgaggctcc	7 (13445)
cgtcctgcag/	GTGATC	Ex 8 (G)	(217)	TCGTCG/	gtgagtccgg	8 (2826)
tcgcttccag/	GAACCA	Ex 9 (H)	(290)	CTGAAG/	gtagcgtggg	9 (5000+)
ctgctgccag/	ACCATC	Ex 10 (I)	(227)	CAAGGG/	gtaagtgttt	10 (1295)
tgccttccag/	CTACAT	Ex 11 (J)	(185)	TGCTGG/	gtgagggccg	11 (2068)
gttcatgcag/	GTCAGG	Ex 12 (K)	(324)	GCAGCC/	gtaagtgcct	12 (2005)
cctcctctag/	CGCCCA	Ex 13 (L)	(200)	ACCCAG/	gcaggtgccc	13 (6963)
tgtcttacag/	CCCTTT	Ex 14 (M)	(209)	GCGAGG/	gtaggaggcc	14 (1405)
cctcccgcag/	GTACCT	Ex 15 (N)	(191)	TGTCAG/	gtaaggggcc	15 (686)
ctgcttgcag/	GGGCCA	Ex 16 (O)	(210)	AGTTCT/	gtacgtgggg	16 (3894)
gtctttgcag/	CAGCCC	Ex 17 (P)	(126)	GTGGAG/	gtaggtgtga	17 (3903)
cctcccccag/	AGCCGC	Ex 18 (Q)	(237)	GTGACG/	gtgaggccct	18 (3042)
tcccttgcag/	CCATCT	Ex 19 (R)	(111)	TGTGTG/	gtgagccagc	19 (1448)
tctctggcag/	AAATCA	Ex 20 (S)	(237)	TCACAG/	gtaaggagcc	20 (1095)
tccctgccag/	GCATCG	Ex 21 (T)	(140)	CCGCCG/	gtgaggggcg	21 (6514)
ctctcctcag/	ATCCTG	Ex 22 (U)	(98)	GTACAG/	gtaggacatc	22 (2275)
tcccttcag/	GCCCTA	Ex 23 (V)	(>262)			23 (19985)

Table 4 page 1 of 7

LRP-5 Exon primers

E1x1 1f	CAGGGTTTCATCCTTTGTGG
E1x1 1fU	TGTAAAACGACGGCCAGTCAGGGTTTCATCCTTTGTGG
E1x1 1fR	GCTATGACCATGATTACGCCCAGGGTTTCATCCTTTGTGG
E1x1 1r	TGACGGGAAGAGTTCCTCAG
E1x1 1rR	GCTATGACCATGATTACGCCTGACGGGAAGAGTTCCTCAG
E1x5 1f	TCTGCTCTTCCTGAACTGCC
E1x5 1fU	TGTAAAACGACGGCCAGTTCTGCTCTTCCTGAACTGCC
E1x5 1r	TTGAGTCCTTCAACAAGCCC
E1x5 1rR	GCTATGACCATGATTACGCCTTGAGTCCTTCAACAAGCCC
E1x6 1fU	TGTAAAACGACGGCCAGTTTCCCCACTCATAGAGGCTC
E1x6 1rR	GCTATGACCATGATTACGCCGCTCCCAACTCGCCAAGT
E1x6a 1fU	TGTAAAACGACGGCCAGTGGTCAACATGGAGGCAGC
E1x6a 1rR	GCTATGACCATGATTACGCCCAGGTGTCAGTCCGCTTG
E1x6b 1fU	TGTAAAACGACGGCCAGTGCAGAGAAGTTCTGAGC
E1x6b 1rR	GCTATGACCATGATTACGCCCACTTGGCCAGCCATACTC
E1x6c 1fU	TGTAAAACGACGGCCAGTCAAGCAAGCCTCTTGCTACC
E1x6c 1rR	GCTATGACCATGATTACGCCACTGCAATGAGGTGAAAGGC
E1x6d 1fU	TGTAAAACGACGGCCAGTCAGGTGAGAACAAGTGTCCG
E1x6d 1rR	GCTATGACCATGATTACGCCGCTGCCTCCATGTTGACC
E1x6e 1fU	TGTAAAACGACGGCCAGTTGTGCCTGGGTGAGATTCT
E1x6e 1rR	GCTATGACCATGATTACGCCTGTGGAGCCTCTATGAGTGG
E1x6f 1fU	TGTAAAACGACGGCCAGTGGGTGACAGGTGGCAGTAG
E1x6f 1rR	GCTATGACCATGATTACGCCGGAAGGAAGGACACTTGAGC
E1x6g 1fU	TGTAAAACGACGGCCAGTCCTGGTGTGTTTGAGAACCC
E1x6g 1rR	GCTATGACCATGATTACGCCCAATGGGAAGCCAGGCTAG
E1xA 1f	ATCTTGCTGGCTTAGCCAGT
E1xA 1fU	TGTAAAACGACGGCCAGTATCTTGCTGGCTTAGCCAGT
E1xA 1fR	GCTATGACCATGATTACGCCATCTTGCTGGCTTAGCCAGT
E1xA 1r	GCTCATGCAAATTCGAGAGAG
E1xA 1rR	GCTATGACCATGATTACGCCGCTCATGCAAATTCGAGAGAG
E1xB 1f	CCTGTTGGTTATTTCCGATGG
E1xB 1fU	TGTAAAACGACGGCCAGTCCTGTTGGTTATTTCCGATGG
E1xB 1fR	GCTATGACCATGATTACGCCCCTGTTGGTTATTTCCGATGG
E1xB 1r	CCTGAGTTAAGAAGGAACGCC
E1xB 1rR	GCTATGACCATGATTACGCCCCTGAGTTAAGAAGGAACGCC
E1xC 1f	AATTGGGTCAGCAGCAATG

Table 4 page 2 of 7 con't.

E1xC 1fR	GCTATGACCATGATTACGCCAATTGGGTCAGCAGCAATG
E1xC 2f	AATTGGGTCAGCAGCAATG
E1xC 2fU	TGTAAAACGACGGCCAGTAATTGGGTCAGCAGCAATG
E1xC 2fR	GCTATGACCATGATTACGCCAATTGGGTCAGCAGCAATG
E1xC 1r	TTGGATCGCTAGAGATTGGG
E1xC 1rR	GCTATGACCATGATTACGCCTTGGATCGCTAGAGATTGGG
E1xC 2r	GCACCCTAATTGGCACTCA
E1xC 2rR	GCTATGACCATGATTACGCCGCACCCTAATTGGCACTCA
E1xD 1f	TGACGGTCCTCTTCTGGAAC
E1xD 1fR	GCTATGACCATGATTACGCCTGACGGTCCTCTTCTGGAAC
E1xD 2f	CGAGGCAGGATGTGACTCAT
E1xD 2fU	TGTAAAACGACGGCCAGTCGAGGCAGGATGTGACTCAT
E1xD 2fR	GCTATGACCATGATTACGCCCGAGGCAGGATGTGACTCAT
E1xD 1r	AGTGGATCATTTCGAACGG
E1xD 1rR	GCTATGACCATGATTACGCCAGTGGATCATTTCGAACGG
E1xD 2r	CCAACTCAGCTTCCCGAGTA
E1xD 2rR	GCTATGACCATGATTACGCCCCAACTCAGCTTCCCGAGTA
E1xE 1f	TGGCTGAGTATTTCCCTTGC
E1xE 1fU	TGTAAAACGACGGCCAGTTGGCTGAGTATTTCCCTTGC
E1xE 1fR	GCTATGACCATGATTACGCCTGGCTGAGTATTTCCCTTGC
E1xE 1r	TTTAACAAGCCCTCCTCCG
E1xE 1rR	GCTATGACCATGATTACGCCTTTAACAAGCCCTCCTCCG
E1xF 1f	CAACGCCAGCATCTACTGA
E1xF 1fU	TGTAAAACGACGGCCAGTCAACGCCAGCATCTACTGA
E1xF 1fR	GCTATGACCATGATTACGCCCAACGCCAGCATCTACTGA
E1xF 1r	CAAATAGCAGAGCACAGGCA
E1xF 1rR	GCTATGACCATGATTACGCCCAAATAGCAGAGCACAGGCA
E1xG 1f	TGAAGTTGCTGCTCTTGGG
E1xG 1fU	TGTAAAACGACGGCCAGTTGAAGTTGCTGCTCTTGGG
E1xG 1fR	GCTATGACCATGATTACGCCTGAAGTTGCTGCTCTTGGG
E1xG 1r	CACTTCCTCCTCATGCAAGTC
E1xG 1rR	GCTATGACCATGATTACGCCCACTTCCTCCTCATGCAAGTC
E1xH 1f	AGACTGGAGCCTCTGTGTTCG
E1xH 1fU	TGTAAAACGACGGCCAGTAGACTGGAGCCTCTGTGTTCG
E1xH 1fR	GCTATGACCATGATTACGCCAGACTGGAGCCTCTGTGTTCG
E1xH 1r	TGTGTGTCTACCGGACTTGC
E1xH 1rR	GCTATGACCATGATTACGCCTGTGTGTCTACCGGACTTGC
E1xH 2r	GAACAGAGGCAAGGTTTTCCC
E1xH 2rR	GCTATGACCATGATTACGCCGAACAGAGGCAAGGTTTTCCC
E1xI 1f	AGAATCGCTTGAACCCAGG
E1xI 1fR	GCTATGACCATGATTACGCCAGAATCGCTTGAACCCAGG
E1xI 2f	GCTGGTTCCTAAAATGTGGC
E1xI 2fU	TGTAAAACGACGGCCAGTGCTGGTTCCTAAAATGTGGC
E1xI 2fR	GCTATGACCATGATTACGCCGCTGGTTCCTAAAATGTGGC

Table 4 page 3 of 7 con't.

E1xI 1r	CATACGAGGTGAACACAAGGAC
E1xI 1rR	GCTATGACCATGATTACGCCCATACGAGGTGAACACAAGGAC
E1xJ 1f	TGAAGAGGTGGGGACAGTTG
E1xJ 1fR	GCTATGACCATGATTACGCCTGAAGAGGTGGGGACAGTTG
E1xJ 2f	CTTGTGCCTTCCAGCTACATC
E1xJ 2fU	TGTAAAACGACGGCCAGTCTTGTGCCTTCCAGCTACATC
E1xJ 2fR	GCTATGACCATGATTACGCCCTTGTGCCTTCCAGCTACATC
E1xJ 1r	AGTCCTGGCACAGGGATTAG
E1xJ 1rR	GCTATGACCATGATTACGCCAGTCCTGGCACAGGGATTAG
E1xJ 2r	ATAACTGCAGCAAAGGCACC
E1xJ 2rR	GCTATGACCATGATTACGCCATAACTGCAGCAAAGGCACC
E1xK 1f	GCTTCAGTGGATCTTGCTGG
E1xK 1fU	TGTAAAACGACGGCCAGTGCTTCAGTGGATCTTGCTGG
E1xK 1fR	GCTATGACCATGATTACGCCGCTTCAGTGGATCTTGCTGG
E1xK 1r	TGTGCAGTGCACAACCTACC
E1xK 1rR	GCTATGACCATGATTACGCCTGTGCAGTGCACAACCTACC
E1xL 1f	GTTGTCGAGTGGCGTGCTAT
E1xL 1fU	TGTAAAACGACGGCCAGTGTTGTCGAGTGGCGTGCTAT
E1xL 1fR	GCTATGACCATGATTACGCCGTTGTCGAGTGGCGTGCTAT
E1xL 1r	AAAAGTCCTGTGGGGTCTGA
E1xL 1rR	GCTATGACCATGATTACGCCAAAAGTCCTGTGGGGTCTGA
E1xM 1f	AGAAGTGTGGCCTCTGCTGT
E1xM 1fU	TGTAAAACGACGGCCAGTAGAAGTGTGGCCTCTGCTGT
E1xM 1fR	GCTATGACCATGATTACGCCAGAAGTGTGGCCTCTGCTGT
E1xM 1r	GTGAAAGAGCCTGTGTTTGCT
E1xM 1rR	GCTATGACCATGATTACGCCGTGAAAGAGCCTGTGTTTGCT
E1xN 1f	AGACCCTGCTTCCAAATAAGC
E1xN 1fU	TGTAAAACGACGGCCAGTAGACCCTGCTTCCAAATAAGC
E1xN 1fR	GCTATGACCATGATTACGCCAGACCCTGCTTCCAAATAAGC
E1xN 1r	ACTCATTTTCTGCCTCTGCC
E1xN 1rR	GCTATGACCATGATTACGCCACTCATTTTCTGCCTCTGCC
E1xO 1f	TGGCAGTCCTGTCAACCTCT
E1xO 1fU	TGTAAAACGACGGCCAGTTGGCAGTCCTGTCAACCTCT
E1xO 1fR	GCTATGACCATGATTACGCCTGGCAGTCCTGTCAACCTCT
E1xO 1r	CACACAGGATCTTGCACTGG
E1xO 1rR	GCTATGACCATGATTACGCCCACACAGGATCTTGCACTGG
E1xP 1f	AGGGCCAGTTCTCATGAGTT
E1xP 1fU	TGTAAAACGACGGCCAGTAGGGCCAGTTCTCATGAGTT
E1xP 1fR	GCTATGACCATGATTACGCCAGGGCCAGTTCTCATGAGTT
E1xP 1r	GGGCAAAGGAAGACACAATC
E1xP 1rR	GCTATGACCATGATTACGCCGGGCAAAGGAAGACACAATC
E1xQ 1f	CAACTTCTGCTTTGAAGCCC

Table 4 page 4 of 7 con't.

E1xQ 1fU	TGTAAAACGACGGCCAGTCAACTTCTGCTTTGAAGCCC
E1xQ 1fR	GCTATGACCATGATTACGCCCAACTTCTGCTTTGAAGCCC
E1xQ 1r	GACAGACTTGGCAATCTCCC
E1xQ 1rR	GCTATGACCATGATTACGCCGACAGACTTGGCAATCTCCC
E1xR 1f	TCTGCTCTCTGTTTGGAGTCC
E1xR 1fU	TGTAAAACGACGGCCAGTTCTGCTCTCTGTTTGGAGTCC
E1xR 1fR	GCTATGACCATGATTACGCCTCTGCTCTCTGTTTGGAGTCC
E1xR 1r	CCCTAAACTCCACGTTCCTG
E1xR 1rR	GCTATGACCATGATTACGCCCCCTAAACTCCACGTTCCTG
E1xS 1f	GGGTTAATGTTGGCCACATC
E1xS 1fR	GCTATGACCATGATTACGCCGGGTTAATGTTGGCCACATC
E1xS 2f	TTGGCAGGGATGTGTTGAG
E1xS 2fU	TGTAAAACGACGGCCAGTTTGGCAGGGATGTGTTGAG
E1xS 2fR	GCTATGACCATGATTACGCCTTGGCAGGGATGTGTTGAG
E1xS 1r	GTCTGCCACATGTGCAAGAG
E1xS 1rR	GCTATGACCATGATTACGCCGTCTGCCACATGTGCAAGAG
E1xT 1f	TGGTCTGAGTCTCGTGGGTA
E1xT 1fU	TGTAAAACGACGGCCAGTTGGTCTGAGTCTCGTGGGTA
E1xT 1fR	GCTATGACCATGATTACGCCTGGTCTGAGTCTCGTGGGTA
E1xT 1r	GAGGTGGATTTGGGTGAGATT
E1xT 1rR	GCTATGACCATGATTACGCCGAGGTGGATTTGGGTGAGATT
E1xU 1f	AGCCCTCTCTGCAAGGAAAG
E1xU 1fU	TGTAAAACGACGGCCAGTAGCCCTCTCTGCAAGGAAAG
E1xU 1fR	GCTATGACCATGATTACGCCAGCCCTCTCTGCAAGGAAAG
E1xU 1r	CAGAACGTGGAGTTCTGCTG
E1xU 1rR	GCTATGACCATGATTACGCCCAGAACGTGGAGTTCTGCTG
E1xV 1f	TACCGAATCCCACTCCTCTG
E1xV 1fU	TGTAAAACGACGGCCAGTTACCGAATCCCACTCCTCTG
E1xV 1fR	GCTATGACCATGATTACGCCTACCGAATCCCACTCCTCTG
E1xV 2f	CATGGTAGAGGTGGGACCAT
E1xV 2fU	TGTAAAACGACGGCCAGTCATGGTAGAGGTGGGACCAT
E1xV 2fR	GCTATGACCATGATTACGCCCATGGTAGAGGTGGGACCAT
E1xV 1r	GATATCCACCTCTGCCCAAG
E1xV 1rR	GCTATGACCATGATTACGCCGATATCCACCTCTGCCCAAG
E1xV 2r	TTACAGGGGCACAGAGAAGC
E1xV 2rR	GCTATGACCATGATTACGCCTTACAGGGGCACAGAGAAGC

Table 4 page 5 of 7 con't.

SNP primers

57-1 1f	GCAACAGAGCAAGACCCTGT
57-1 1fR	GCTATGACCATGATTACGCCGCAACAGAGCAAGACCCTGT
57-1 1r	AAATTAGCCAGGCATGGTG
57-1 1rR	GCTATGACCATGATTACGCCAAATTAGCCAGGCATGGTG
57-1 1fU	TGTAAAACGACGGCCAGTGCAACAGAGCAAGACCCTGT
57-2 1f	CCTGCAGAAGGAAACCTGAC
57-2 1fR	GCTATGACCATGATTACGCCCCTGCAGAAGGAAACCTGAC
57-2 1r	CTGCATCTTTGCCACCATG
57-2 1rR	GCTATGACCATGATTACGCCCTGCATCTTTGCCACCATG
57-2 1fU	TGTAAAACGACGGCCAGTCCTGCAGAAGGAAACCTGAC
57-3 1f	TTCCCAGGAGGCAAGTTATG
57-3 1fR	GCTATGACCATGATTACGCCTTCCCAGGAGGCAAGTTATG
57-3 1r	TGGGCTTAGGTGATCCTCAC
57-3 1rR	GCTATGACCATGATTACGCCTGGGCTTAGGTGATCCTCAC
57-3 1fU	TGTAAAACGACGGCCAGTTTCCCAGGAGGCAAGTTATG
57-4 1f	ACCAAGCCCAACTAATCAGC
57-4 1fR	GCTATGACCATGATTACGCCACCAAGCCCAACTAATCAGC
57-4 1r	ATGCCTGTAATCCCAGCACT
57-4 1rR	GCTATGACCATGATTACGCCATGCCTGTAATCCCAGCACT
57-4 1fU	TGTAAAACGACGGCCAGTACCAAGCCCAACTAATCAGC
57-5 1f	ACTGCAAGCCCTCTCTGAAC
57-5 1r	CGAAGACTGCGAAACAGACA
58-1 1f	CTAGTGCCGTGCAGAATGAG
58-1 1r	GGCCACTGCAATGAGATACA
58-2 1f	GAGAAACAGTTCCAGGGTGG
58-2 1fR	GCTATGACCATGATTACGCCGAGAAACAGTTCCAGGGTGG
58-2 1r	AAACTGAGGCTGGGAGAGGT
58-2 1rR	GCTATGACCATGATTACGCCAAACTGAGGCTGGGAGAGGT
58-3 1f	TGTTCTTCCTCACAGGGAGG
58-3 1fR	GCTATGACCATGATTACGCCTGTTCTTCCTCACAGGGAGG
58-3 1r	TCCCCAAATCTGTCCAGTTC
58-3 1rR	GCTATGACCATGATTACGCCTCCCCAAATCTGTCCAGTTC
58-4 1f	CATACCTGGAGGGATGCTTG
58-4 1fR	GCTATGACCATGATTACGCCCATACCTGGAGGGATGCTTG
58-4 1r	TAGGTTGCTGTGTGGCTTCA
58-4 1rR	GCTATGACCATGATTACGCCTAGGTTGCTGTGTGGCTTCA
58-5 1f	CTTCTGACAAAGCAGAGGCC
58-5 1fR	GCTATGACCATGATTACGCCCTTCTGACAAAGCAGAGGCC

Table 4 page 6 of 7 con't.

58-5 1r	GCTGTTAGGGTTACCATCGC
58-5 1rR	GCTATGACCATGATTACGCCGCTGTTAGGGTTACCATCGC
58-6 1f	CCACAGGGTGATATGCTGTC
58-6 1fR	GCTATGACCATGATTACGCCCCACAGGGTGATATGCTGTC
58-6 1r	CGCCTGGCTACTTTGGTACT
58-6 1rR	GCTATGACCATGATTACGCCCGCCTGGCTACTTTGGTACT
58-7 1f	CCAAATGAACCTGGGCAAC
58-7 1fR	GCTATGACCATGATTACGCCCCAAATGAACCTGGGCAAC
58-7 1r	GTCTTGGCTCACTGCAACCT
58-7 1rR	GCTATGACCATGATTACGCCGTCTTGGCTCACTGCAACCT
58-8 1f	GCCAAGACTGTGCTACTGCA
58-8 1r	CAGGGAGCAGATCTTACCCA
58-9 1f	TGGGATTAACTAGGGAGGGG
58-9 1fR	GCTATGACCATGATTACGCCTGGGATTAACTAGGGAGGGG
58-9 1r	TGCTGCTGTCTCCATCTCTG
58-9 1rR	GCTATGACCATGATTACGCCTGCTGCTGTCTCCATCTCTG
58-10 1f	ACAGACCAGCAGTGAAACCTG
58-10 1fR	GCTATGACCATGATTACGCCACAGACCAGCAGTGAAACCTG
58-10 1r	GTTCACTGCAACCTCTGCCT
58-10 1rR	GCTATGACCATGATTACGCCGTTCACTGCAACCTCTGCCT
58-11 1f	GTTCTCGTAGATGCTTGCAGG
58-11 1fR	GCTATGACCATGATTACGCCGTTCTCGTAGATGCTTGCAGG
58-11 1r	GAGGCAGGAGGATCACTTGA
58-11 1rR	GCTATGACCATGATTACGCCGAGGCAGGAGGATCACTTGA
58-12 1f	TGAGCTGAGATCACACCGCT
58-12 1fR	GCTATGACCATGATTACGCCTGAGCTGAGATCACACCGCT
58-12 1r	AGTTGACACTTTGCTGGCCT
58-12 1rR	GCTATGACCATGATTACGCCAGTTGACACTTTGCTGGCCT
58-13 1f	CTCTGCATGGCTTAGGGACA
58-13 1fR	GCTATGACCATGATTACGCCCTCTGCATGGCTTAGGGACA
58-13 1r	GGCTGCTCTCTGCATTCTCT
58-13 1rR	GCTATGACCATGATTACGCCGGCTGCTCTCTGCATTCTCT
58-14 1f	CTGGCTTTAGCTTGCATTTCC
58-14 1fR	GCTATGACCATGATTACGCCCTGGCTTTAGCTTGCATTTCC
58-14 1r	TGCCTCAGTTTTCTCACCTGT
58-14 1rR	GCTATGACCATGATTACGCCTGCCTCAGTTTTCTCACCTGT
58-15 1f	CAAACAGCCACTGAGCATGT
58-15 1fR	GCTATGACCATGATTACGCCCAAACAGCCACTGAGCATGT
58-15 1r	TCCTCCTGTAGATGCCCAAG

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58-15 1rR GCTATGACCATGATTACGCCTCCTCCTGTAGATGCCCAAG

Table 5

LRP-5 exon SNPs

Exon	Polymorphism	Amino Acid Change	Location
exon E	G to A	Intronic	10 bp 3' of exon E
exon E	C to T	none	Phe^{331}, exon E
exon F	G to A	Intronic	50 bp 5' of exon F
exon G	C to T	none	Phe^{518}, exon G
exon I	C to T	none	Asn^{709}, exon I
exon P	C to T	Intronic	82 bp 5' of exon P
exon N	C to T	none	Asp^{1068}, exon N
exon N	A to G	none	Val^{1088}, exon N
exon Q	C to T	Ala^{1299} to Val	Ala^{1299}, exon Q
exon U	T to C	Val^{1494} to Ala	Val^{1494}, exon U

Table 6
SNP's Identified in the IDDM 4 Locus

List of PCR Fragments and available RFLP Sites for Analysis:

PCR Product	SNP	Location	Enzyme
Contig 57			
57-1	a/t	13363	none
57-1	a/g	13484	Bst XI
57-2	a/g	14490	none
57-2	a/g	14885	none
57-3	c/g	18776	Mae II
57-3	t/c	18901	Msp I
57-3	a/g	19313	Afl II
57-4	22T/25T	20800	none
57-5	g/a	23713	Msp I
Contig 58			
58-15	c/t	3015	none
58-14	g/c	3897	Pfl MI
58-13	c/g	5574	Eco NI
58-12	t/g	6051	none
58-11	a/g	8168	none
58-10	a/g	8797	none
58-9	g/t	9445	none
58-9	c/t	9718	none
58-8	insert T	10926	Pst I
58-7	t/a	11449	Bst XI
58-7	t/c	11468	none
58-6	t/c	11878	none
58-6	g/a	12057	none
58-6	a/g	12180	Hga I
58-5	c/t	14073	none
58-4	a/g	15044	Mae II
58-4	t/c	15354	none
58-3	insert G	16325	none
58-2	g/a	17662	none
58-1	g/t	18439	Bgl II

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SNP primers

57-1 1f	GCAACAGAGCAAGACCCTGT
57-1 1fR	GCTATGACCATGATTACGCCGCAACAGAGCAAGACCCTGT
57-1 1r	AAATTAGCCAGGCATGGTG
57-1 1rR	GCTATGACCATGATTACGCCAAATTAGCCAGGCATGGTG
57-1 1fU	TGTAAAACGACGGCCAGTGCAACAGAGCAAGACCCTGT
57-2 1f	CCTGCAGAAGGAAACCTGAC
57-2 1fR	GCTATGACCATGATTACGCCCCTGCAGAAGGAAACCTGAC
57-2 1r	CTGCATCTTTGCCACCATG
57-2 1rR	GCTATGACCATGATTACGCCCTGCATCTTTGCCACCATG
57-2 1fU	TGTAAAACGACGGCCAGTCCTGCAGAAGGAAACCTGAC
57-3 1f	TTCCCAGGAGGCAAGTTATG
57-3 1fR	GCTATGACCATGATTACGCCTTCCCAGGAGGCAAGTTATG
57-3 1r	TGGGCTTAGGTGATCCTCAC
57-3 1rR	GCTATGACCATGATTACGCCTGGGCTTAGGTGATCCTCAC
57-3 1fU	TGTAAAACGACGGCCAGTTTCCCAGGAGGCAAGTTATG
57-4 1f	ACCAAGCCCAACTAATCAGC
57-4 1fR	GCTATGACCATGATTACGCCACCAAGCCCAACTAATCAGC
57-4 1r	ATGCCTGTAATCCCAGCACT
57-4 1rR	GCTATGACCATGATTACGCCATGCCTGTAATCCCAGCACT
57-4 1fU	TGTAAAACGACGGCCAGTACCAAGCCCAACTAATCAGC
57-5 1f	ACTGCAAGCCCTCTCTGAAC
57-5 1r	CGAAGACTGCGAAACAGACA
58-1 1f	CTAGTGCCGTGCAGAATGAG
58-1 1r	GGCCACTGCAATGAGATACA
58-2 1f	GAGAAACAGTTCCAGGGTGG
58-2 1fR	GCTATGACCATGATTACGCCGAGAAACAGTTCCAGGGTGG
58-2 1r	AAACTGAGGCTGGGAGAGGT
58-2 1rR	GCTATGACCATGATTACGCCAAACTGAGGCTGGGAGAGGT
58-3 1f	TGTTCTTCCTCACAGGGAGG
58-3 1fR	GCTATGACCATGATTACGCCTGTTCTTCCTCACAGGGAGG
58-3 1r	TCCCCAAATCTGTCCAGTTC
58-3 1rR	GCTATGACCATGATTACGCCTCCCCAAATCTGTCCAGTTC
58-4 1f	CATACCTGGAGGGATGCTTG
58-4 1fR	GCTATGACCATGATTACGCCCATACCTGGAGGGATGCTTG
58-4 1r	TAGGTTGCTGTGTGGCTTCA
58-4 1rR	GCTATGACCATGATTACGCCTAGGTTGCTGTGTGGCTTCA
58-5 1f	CTTCTGACAAAGCAGAGGCC
58-5 1fR	GCTATGACCATGATTACGCCCTTCTGACAAAGCAGAGGCC

Table 7 page 2 of 3 con't.

58-5 1r	GCTGTTAGGGTTACCATCGC
58-5 1rR	GCTATGACCATGATTACGCCGCTGTTAGGGTTACCATCGC
58-6 1f	CCACAGGGTGATATGCTGTC
58-6 1fR	GCTATGACCATGATTACGCCCCACAGGGTGATATGCTGTC
58-6 1r	CGCCTGGCTACTTTGGTACT
58-6 1rR	GCTATGACCATGATTACGCCCGCCTGGCTACTTTGGTACT
58-7 1f	CCAAATGAACCTGGGCAAC
58-7 1fR	GCTATGACCATGATTACGCCCCAAATGAACCTGGGCAAC
58-7 1r	GTCTTGGCTCACTGCAACCT
58-7 1rR	GCTATGACCATGATTACGCCGTCTTGGCTCACTGCAACCT
58-8 1f	GCCAAGACTGTGCTACTGCA
58-8 1r	CAGGGAGCAGATCTTACCCA
58-9 1f	TGGGATTAACTAGGGAGGGG
58-9 1fR	GCTATGACCATGATTACGCCTGGGATTAACTAGGGAGGGG
58-9 1r	TGCTGCTGTCTCCATCTCTG
58-9 1rR	GCTATGACCATGATTACGCCTGCTGCTGTCTCCATCTCTG
58-10 1f	ACAGACCAGCAGTGAAACCTG
58-10 1fR	GCTATGACCATGATTACGCCACAGACCAGCAGTGAAACCTG
58-10 1r	GTTCACTGCAACCTCTGCCT
58-10 1rR	GCTATGACCATGATTACGCCGTTCACTGCAACCTCTGCCT
58-11 1f	GTTCTCGTAGATGCTTGCAGG
58-11 1fR	GCTATGACCATGATTACGCCGTTCTCGTAGATGCTTGCAGG
58-11 1r	GAGGCAGGAGGATCACTTGA
58-11 1rR	GCTATGACCATGATTACGCCGAGGCAGGAGGATCACTTGA
58-12 1f	TGAGCTGAGATCACACCGCT
58-12 1fR	GCTATGACCATGATTACGCCTGAGCTGAGATCACACCGCT
58-12 1r	AGTTGACACTTTGCTGGCCT
58-12 1rR	GCTATGACCATGATTACGCCAGTTGACACTTTGCTGGCCT
58-13 1f	CTCTGCATGGCTTAGGGACA
58-13 1fR	GCTATGACCATGATTACGCCCTCTGCATGGCTTAGGGACA
58-13 1r	GGCTGCTCTCTGCATTCTCT
58-13 1rR	GCTATGACCATGATTACGCCGGCTGCTCTCTGCATTCTCT
58-14 1f	CTGGCTTTAGCTTGCATTTCC
58-14 1fR	GCTATGACCATGATTACGCCCTGGCTTTAGCTTGCATTTCC
58-14 1r	TGCCTCAGTTTTCTCACCTGT
58-14 1rR	GCTATGACCATGATTACGCCTGCCTCAGTTTTCTCACCTGT
58-15 1f	CAAACAGCCACTGAGCATGT
58-15 1fR	GCTATGACCATGATTACGCCCAAACAGCCACTGAGCATGT
58-15 1r	TCCTCCTGTAGATGCCCAAG

Table 7 page 3 of 3 con't.

58-15 1rR GCTATGACCATGATTACGCCTCCTCCTGTAGATGCCCAAG

TABLE 8

Primers designed by microsatellite rescue for genotyping and restriction mapping of the IDDM4 region on chromosome 11q13. The other primers used are published, and are also in the Genome Database.

255CA3F	GCCGAGAATTGTCATCTTAACT
255CA3R	GGATTGAAAGCTGCAAACTACA
255CA5F	GGAGCCACCACATCCAGTTA
255CA5R	TGGAGGGATTGCTTGAGG
255CA6F	AGGTGTACACCACCATGCCT
255CA6R	TGGTGCCAATTATTGCTGC
14LCA5F	AGATCTTATACACATGTGCGCG
14LCA5R	AGGTGACATCACTTACAGCGG
L15CA1F	ATTACCCAGGCATGGTGC
L15CA1R	CAGGCACTTCTTCCAGGTCT
18018ACF	AGGGTTACACTGGAGTTTGC
18018ACR	AAACCTTCAATGTGTTCATTAAAAC
E0864CAF	TCAACTTTATTGGGGGTTTA
E0864CAR	AAGGTAAAAGTCCAAAATGG
H0570POLYAF	GGACAGTCAGTTATTGAAATG
H0560POLYAR	TTTCCTCTCTGGGAGTCTCT

E0864CA was obtained from the cosmid E0864
H0570POLYA was obtained from the cosmid H0570
255CA5, 255CA3 and 255CA6 were obtained from the PAC255_m_19
14LCA5 and L15CA1 were obtained from the BAC 14_1_15
18018AC was obtained from the PAC 18_o_18

TABLE 9 PCR Primers for obtaining *LRP-3* cDNA

A.) Primers located within human*LRP-3* cDNA:
The primers are numbered beginning at nucleotide 1 in Fig. 17 (a)

1F (muex 1f)
ATGGAGCCCGAGTGAGC

200f
TCAAGCTGGAGTCCACCATC

218R (27R)
ATGGTGGACTCCAGCTTGAC

256F (1F)
TTCCAGTTTTCCAAGGGAG

265R (26R)
AAAACTGGAAGTCCACTGCG

318R (4R)
GGTCTGCTTGATGGCCTC

343F (2F)
GTGCAGAACGTGGTCATCT

361R (21R)
GTGCAGAACGTGGTCATCT

622R (2R)
AGTCCACAATGATCTTCCGG

638F (4F)
CCAATGGACTGACCATCGAC

657R (1R)
GTCGATGGTCAGTCCATTGG

936f
CACTCGCTGTGAGGAGGAC

956R (22R)
TTGTCCTCCTCACAGCGAG

TABLE 9 (Continued - Page 2 of 7)

1040f (51f)
ACAACGGCAGGACGTGTAAG

1174f (40f)
ATTGCCATCGACTACGACC

1277f (52f)
TGGTCAACACCGAGATCAAC

1333f
AACCTCTACTGGACCGACAC

1462f (41f)
CTCATGTACTGGACAGACT

1481R (23R)
CAGTCTGTCCAGTACATGAG

1607f (50f)
GAGACGCCAAGACAGACAAG

1713F (21F)
GGACTTCATCTACTGGACTG

1732r (40r)
CAGTCCAGTAGATGAAGTCC

1904r (k275r)
GTGAAGAAGCACAGGTGGCT

1960r
TCATGTCACTCAGCAGCTCC

1981F (22F)
GCCTTCTTGGTCTTCACCAG

2261F (23F)
GGACCAACAGAATCGAAGTG

2484R (5R)
GTCAATGGTGAGGTCGT

2519F (5F)
ACACCAACATGATCGAGTCG

TABLE 9 (Continued - Page 3 of 7)

2780r
CCGTTGTTGTGCATACAGTC

3011F (24F)
ACAAGTTCATCTACTGGGTG

3154F (25F)
CGGACACTGTTCTGGACGTG

3173R (25R)
CACGTCCAGAACAGTGTCCG

3556R (3R)
TCCAGTAGAGATGCTTGCCA

3577F (3F)
ATCGAGCGTGTGGAGAAGAC

3851r
GTGGCACATGCAAACTGGTC

4094F (30F)
TCCTCATCAAACAGCAGTGC

4173R (6R)
CGGCTTGGTGATTTCACAC

4687F (6F)
GTGTGTGACAGCGACTACAGC

4707R (30R)
GCTGTAGTCGCTGTCACACAC

5061R (7R)
GTACAAAGTTCTCCCAGCCC

3' end with XbaI site
5069r
GCTCTAGAGTACAAAGTTCTCCCAGCCC

Soluble/HSV/His primers
HLRP3_His_primer1 (4203r)
ATCCTCGGGGTCTTCCGGGGCGAGTTCTGGCTGGCTACTGCTGTGGGCCGGGCT

TABLE 9 (Continued - Page 4 of 7)

HLRP3_His_primer2
TGGATATCTCAGTGGTGGTGGTGGTGCTCGACATCCTCGGGGTCTTCCGG
G

HLRP3_5'_primer (49f)
TAGAATTCGCCGCCACCATGGAGGCAGCGCCGCCC

B.) Mouse *Lrp-3* cDNA primers.
The primers are numbered beginning at nucleotide 1 in Figure 18(a).

13f (mulrp3 5f)
GAGGCGGGAGCAAGAGG

68f (MucD 1f)
GC Hind 3 CATGGAGCCCGAGTGAGC

69f (muex 1f)
ATGGAGCCCGAGTGAGC

83r (muex 1r)
TCACTCGGGCTCCATGG

171f (MucD 2f)
TGCTGTACTGCAGCTTGGTC

300f (MucD 10F)
ATGCAGCTGCTGTAGACTTCC

378r (mulrp3 3r)
GTCTGTTTGATGGCCTCCTC

414r (MucD 7R)
ATGTTCTGTGCAGCACCTCC

445r (mulrp3 4r)
GCCATCAGGTGACACGAG

536f (MucD 11F)
AAGGTTCTCTTCTGGCAGGAC

619r (MucD 12R)
CCAGTCAGTCCAGTACATG

TABLE 9 (Continued - Page 5 of 7)

714f (museq 1f)
TCGACCTGGAGGAACAGAAG

752f (mulrpAb 1f)
AAGCTCAGCTTCATCCACCG

765r (MucD 8R)
ATGAAGCTGAGCTTGGCATC

915f (MucD 12F)
AGCAGAGGAAGGAGATCCTTAG

957r (MucD 9R)
TCCATGGGTGAGTACAGAGC

1105r (museq 1r)
ATTGTCCTGCAACTGCACAC

1232f (MucD 13F)
GCCATTGCCATTGACTACG

1254r (MucD 10R)
GGATCGTAGTCAATGGCAATG

1425f (MucD 14F)
GAATTGAGGTGACTCGCCTC

1433r (MucD 18R)
CCTCAATTCTGTAGTGCCTG

1501f (muxt 4f)
TGTGTTGCACCCTGTGATG

1579r (MucD 11R)
ATCTAGGTTGGCGCATTCG

1610r (MucD 13R)
AGGTGTTCACCAGGACATG

1710r (mulrpAb 1r)
GCGAGCTCCCGTCTATGTTGATCACCTCG

1868f (MucD 3f)
GACCTGATGGGACTCAAAGC

TABLE 9 (Continued - Page 6 of 7)

2062r (MucD 2r)
GCTGGTGAATACCAGGAAGG

2103f (MucD 4f)
ACGATGTGGCTATCCCACTC

2422r (MucD 14R)
AGTAGGATCCAGAGCCAGAG

2619f (MucD 5f)
AGCGCATGGTGATAGCTGAC

2718r (MucD 3r)
CGTTCAATGCTATGCAGGTTC

2892f (MucD 15F)
GTGCTTCACACTACACGCTG

2959f (MucD 6f)
CAGCCAGAAATTTGCCATC

3218r (MucD 4r)
TCCGGCTGTAGATGTCAATG

3237f (MucD 7f)
AGGCCACCAACACTATCAATG

3348r (MucD 52R)
TACCCTCGCTCAGCATTGAC

3554f (MucD 8f)
CTGGAAGATGCCAACATCG

3684r (MucD 5r)
TGAACCCTAGTCCGCTTGTC

3848f (MucD 18F)
CTGCAGAACCTGCTGACTTG

3973f (MucD 19F)
CCAGAGTGATGAAGAAGGCTG

3981r (MucD 15R)
TCACTCTGGTCAGCACACTC

TABLE 9 (Continued - Page 7 of 7)

4079f (MucD 16F)
CAGGATCGCTCTGATGAAGC

4105r (MucD 53R)
GCAGTTAGCTTCATCAGAGCG

4234f (MucD 9f)
ACCCTCTGATGACATCCCAG

4270r (MucD 16R)
AATGGCACTGCTGTGGGC

4497r (MucD 6r)
AGGCTCATGGAGCTCATCAC

4589r (MucD 54R)
ATAGTGTGGCCTTTGTGCTG

4703f (MucD 17F)
GTCATTCGAGGTATGGCACC

4799r (MucD 17R)
GGTAGTATTTGCTGCTCTTCC

5114r (MucD 1r)
GC Xba I AAAGTTTCCCAGCCCTGCC

Soluble/adeno primers

3554f (MsolF)
CTGGAAGATGCCAACATCG

4264r (MHisR)
GCTCTAGACTAGTGATGGTGATGGTGATGACTGCTGTGGGCTGGGATGTCATC
AGAGGGTGG

Table 10 Summary of Serum Chemistry Comparison of LRP3 treatment vs control

Variable	Mouse Type	Treatment (%diff± SE)	p-value (Treatment)
triglycerides	WT+KO	-30± 14	0.025
alkaline phosphatase#	WT+KO	-49± 15	0.001
total cholesterol	KO only	-28± 15	0.073
total cholesterol	WT only	30± 13	0.080
AST#	WT+KO	8± 66	0.912
ALT#	WT+KO	-34± 51	0.431
BUN	WT+KO	-19± 15	0.195

statistically significantly higher baseline values for controls.

Table 11: Summary for Blood Chemistry Variables Pooled over Knockout and Wild-Type Mice

Variable	Treat Group	Animal Type	n	baseline (mean±%CV)	post-treat (mean±%CV)t	change	%change (95%CI)	p-value (%chg)
trigly(mg/dL)	Control	POOLED	10	86± 13%	186± 35%	100	115% (61, 189)	<0.001
trigly(mg/dL)	LDL	POOLED	9	92± 31%	81± 55%	-12	-13% (-35, 17)	0.321
trigly(mg/dL)	LRP3	POOLED	8	99± 24%	128± 36%	29	30% (-10, 86)	0.133
alkphos(U/L)	Control	POOLED	10	190± 19%	374± 30%	184	97% (68, 130)	<0.001
alkphos(U/L)	LDL	POOLED	9	162± 12%	193± 29%	31	19% (-1, 43)	0.061
alkphos(U/L)	LRP3	POOLED	8	154± 13%	146± 35%	-8	-5% (-24, 19)	0.604
totchol(mg/dL)	Control	POOLED	10	116± 69%	176± 86%	60	51% (21, 89)	0.002
totchol(mg/dL)	LDL	POOLED	9	124± 58%	87± 68%	-37	-30% (-41, -17)	0.001
totchol(mg/dL)	LRP3	POOLED	8	127± 62%	166± 57%	39	30% (9, 56)	0.009
AST(U/L)	Control	POOLED	9	41± 22%	821± 69%	780	1894% (1142, 3101)	<0.001
AST(U/L)	LDL	POOLED	8	41± 25%	362± 61%	320	772% (369, 1520)	<0.001
AST(U/L)	LRP3	POOLED	8	33± 21%	989± 129%	955	2888% (953, 8380)	<0.001
ALT(U/L)	Control	POOLED	10	33± 15%	624± 59%	591	1798% (1203, 2665)	<0.001
ALT(U/L)	LDL	POOLED	8	32± 36%	331± 42%	299	938% (447, 1872)	<0.001
ALT(U/L)	LRP3	POOLED	8	25± 35%	1020± 157%	994	3944% (861, 16921)	<0.001
BUN(U/L)	Control	POOLED	8	29± 12%	23± 11%	-5	-19% (-29, -7)	0.008
BUN(U/L)	LDL	POOLED	9	28± 19%	25± 14%	-3	-12% (-22, 1)	0.062
BUN(U/L)	LRP3	POOLED	8	28± 12%	19± 41%	-9	-31% (-53, 2)	0.058

Note means given are geometric means.
p-value is from a 2-sided paired t-test.

Table 12 Regions of Sequence Similarity Between Human and Mouse LRP-3

Location in Human Sequence	Nucleotide Length	Percent Identity	BLAST Score	Exon Name
Contig 31				
20235-20271	37	86	140	
24410-24432	23	86	88	
24464-24667	204	82	168, 223	6
24904-24995	52	82	179	
25489-25596	108	81	360	
26027-26078	52	80	170	
26192-26261	70	84	251	
26385-26486	102	87	393	
28952-28993	42	85	156	
41707-41903	197	90	823	
42827-42898	66	81	222	
43468-43585	117	85	316	
50188-50333	146	86	550	
54455-54494	40	80	128	
54718-54750	33	87	129	
59713-60123	411	87	1587	A
78536-78680	145	80	473	D
87496-87548	53	88	211	
87598-87717	120	84	429	
90772-90819	48	85	177	
99457-99795	339	83	1182	E
103094-103281	188	83	661	F
116659 -116954	296	81	985	G
119754-120089	336	83	1167	H
Contig 30				
8920-9256	337	89	1026	K
11238-11353	116	84	*418	L
18394-18648	255	80	825	M
20020-20224	205	84	746	N
20926-21153	228	83	807	O
24955-25155	201	82	672	P
29126-19288	163	74	*437	Q
33874-34033	160	85	*593	S
35205-35340	136	86	509	T
41911-41911	55	80	*176	U
44629-44681	53	73	*249	V

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding a polypeptide which includes the amino acid sequence shown in Figure 5(e).

2. A nucleic acid molecule according to claim 1 wherein the polypeptide includes the amino acid sequence shown in Figure 5(c).

3. A nucleic acid molecule according to claim 2 including the coding sequence shown in Figure 5(a).

4. An isolated nucleic acid molecule encoding a polypeptide and which hybridizes under stringent conditions to nucleic acid according to claim 3.

5. An isolated nucleic acid molecule encoding a polypeptide which is a mutant, allele, variant or derivative of the amino acid sequence of Figure 5(e) or Figure 5(c), by way of addition, deletion, insertion and/or substitution of one or more amino acids.

6. A nucleic acid molecule according to claim 5 wherein said polypeptide includes the amino acid sequence of a polypeptide selected from that shown in Figure 11(c), Figure 12(d) and Figure 18(c).

7. A nucleic acid molecule according to claim 6 including a coding sequence selected from the nucleotide sequences shown in Figure 11(b), Figure 12(d), Figure 13, Figure 14, Figure 15(a) and Figure 18(b).

8. A nucleic acid molecule according to claim 7 wherein the coding sequence is that shown in Figure 11(b), included within a molecule which has the sequence shown in Figure 11(a).

9. An isolated nucleic acid molecule including the sequence

of a nucleic acid molecule according to any preceding claim with an alteration which is associated with IDDM.

10. An isolated nucleic acid molecule including the sequence of a nucleic acid molecule according to any of claims 1 to 8 with an alteration shown in Table 5 or Table 6.

11. An oligonucleotide fragment of a nucleic acid molecule according to any preceding claim of at least about 14 nucleotides.

12. An oligonucleotide with a sequence shown in any of Tables 2, 4, 7, 8 and 9.

13. An isolated nucleic acid molecule including a *LRP-5* gene promoter.

14. A nucleic acid molecule according to claim 13 including a promoter, the promoter including the sequence or nucleotides shown in Figure 12(e) or Figure 15(b).

15. An isolated polypeptide including the amino acid sequence shown in Figure 5(e).

16. A polypeptide according to claim 15 including the amino acid sequence shown in Figure 5(c).

17. An isolated polypeptide which is an amino acid sequence mutant, variant, allele or derivative of the amino acid sequence of Figure 5(e) or Figure 5(c), by way of addition, deletion, insertion and/or substitution of one or more amino acids.

18. A polypeptide according to claim 17 wherein said polypeptide includes the amino acid sequence of a polypeptide selected from that shown in Figure 11(c), Figure 12(d) and Figure 18(c).

19. A fragment of a polypeptide according to any of claims 15 to 18 including at least 5 contiguous amino acids of an amino acid sequence selected from the amino acid sequences of Figure 5(c), Figure 11(c), Figure 12(d) and Figure 18(c).

20. A fragment according to claim 19 which has an amino acid sequence selected from:

SYFHLFPPPPSPCTDSS,
VDGRQNIKRAKDDGT,
EVLFTTGLIRPVALVVDN, and
IQGHLDFVMDILVFHS.

21. A fragment according to claim 19 which includes the LRP5 extracellular domain.

22. A fragment according to claim 19 which includes the LRP5 cytoplasmic domain.

23. A method of production of a polypeptide according to any of claims 15 to 18 which includes expression of the polypeptide from encoding nucleic acid.

24. A method according to claim 23 further including isolating and/or purifying the polypeptide.

25. A method according to claim 23 or claim 24 further including formulating the polypeptide into a composition which includes at least one additional component.

26. A composition including a polypeptide according to any of claims 15 to 18 and a pharmaceutically acceptable excipient.

27. A method of production of a fragment according to any of claims 19 to 22 which includes expression of the fragment from encoding nucleic acid.

28. A method according to claim 27 further including

isolating and/or purifying the polypeptide.

29. A method according to claim 27 or claim 28 further including formulating the polypeptide into a composition which includes at least one additional component.

30. A composition including a fragment of a polypeptide according to any of claims 19 to 22, or a functional mimetic thereof, and a pharmaceutically acceptable excipient.

31. A composition including a nucleic acid molecule according to any of claims 1 to 10 and a pharmaceutically acceptable excipient.

32. An isolated antibody specific for a polypeptide according to any of claims 15 to 18.

33. An isolated antibody according to claim 32 which binds an amino acid sequence selected from:

SYFHLFPPPPSPCTDSS,
VDGRQNIKRAKDDGT,
EVLFTTGLIRPVALVVDN, and
IQGHLDFVMDILVFHS.

34. A composition including an antibody according to claim 32 or claim 33 and a pharmaceutically acceptable excipient.

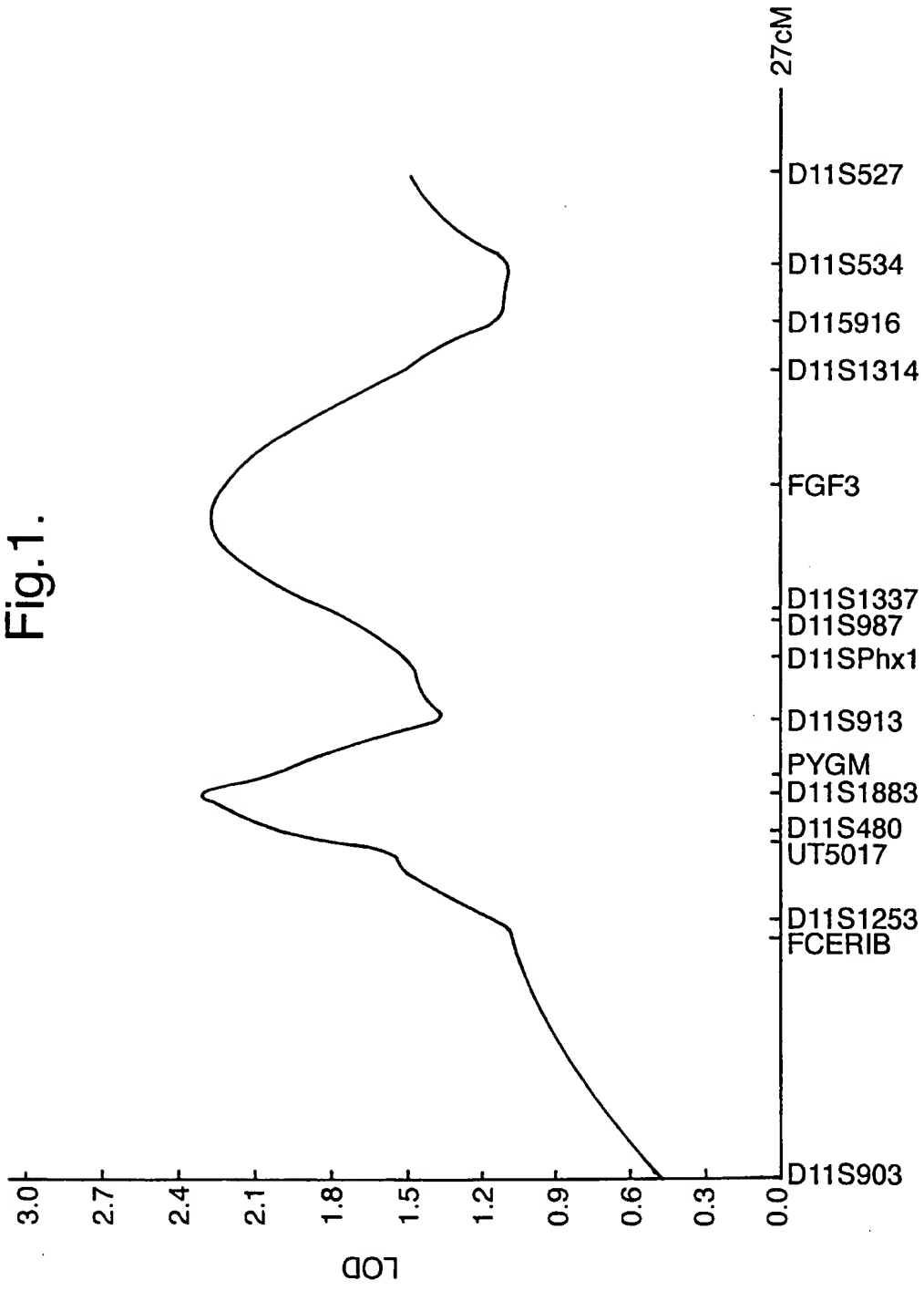
35. A method for determining if an individual is susceptible to IDDM, comprising determining if a nucleic acid selected from the group consisting of the nucleic acids shown in Figure 5(e), Figure 5(c), Figure 5(a), Figure 11(b), Figure 12(d), Figure 13, Figure 14, Figure 15(a) and Figure 15(b) hybridizes with a sample of the individual's DNA.

36. A method including determining the presence or absence in a test sample of a nucleotide sequence selected from those of the nucleic acid molecules according to any of claims 1 to 10.

36. Use of a polypeptide according to any of claims 15 to 18, or nucleic acid encoding a said polypeptide in the manufacture of a medicament for reducing triglyceride levels in serum of an individual.

37. A method of reducing triglyceride levels in serum of an individual, the method including administering to the individual a polypeptide according to any of claims 15 to 18, or nucleic acid encoding a said polypeptide.

Fig.1.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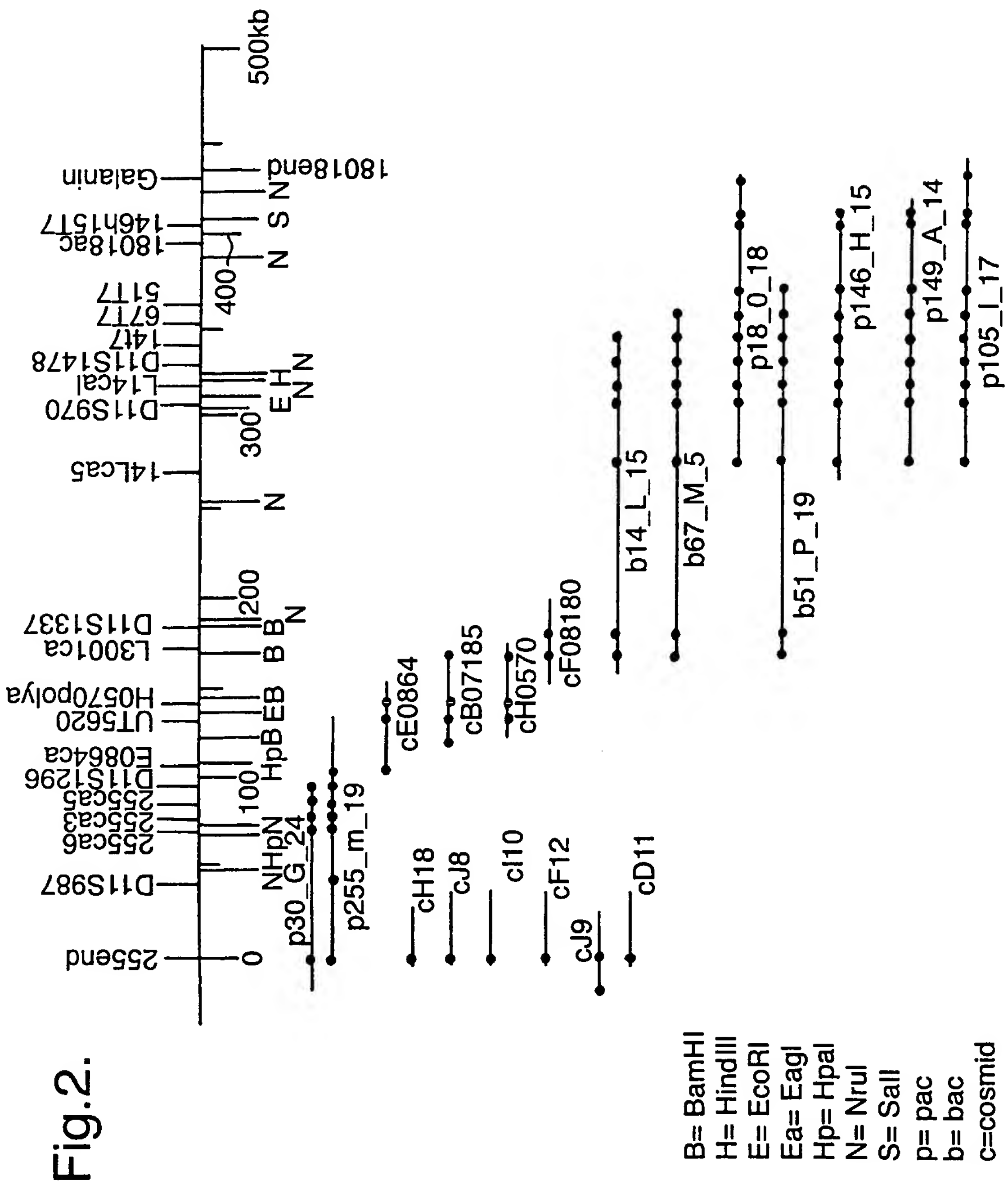
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L3001ca
D11S1337
14Lca5
D11S970
L14cal
D11S1478
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67T7
51T7
18018ac
146h15T7
Galanin
18018end
N S N N H E N N B B N B EB HpB NHpN
p30_G_24
p255_m_19
cH18
cJ8
cI10
cF12
cJ9
cD11
cE0864
cB07185
cH0570
cF08180
b14_L_15
b67_M_5
b51_P_19
p18_0_18
p146_H_15
p149_A_14
p105_I_17
B= BamHI
H= HindIII
E= EcoRI
Ea= EagI
Hp= HpaI
N= NruI
S= SalI
p= pac
b= bac
c=cosmid

Fig.2.

Fig.3.

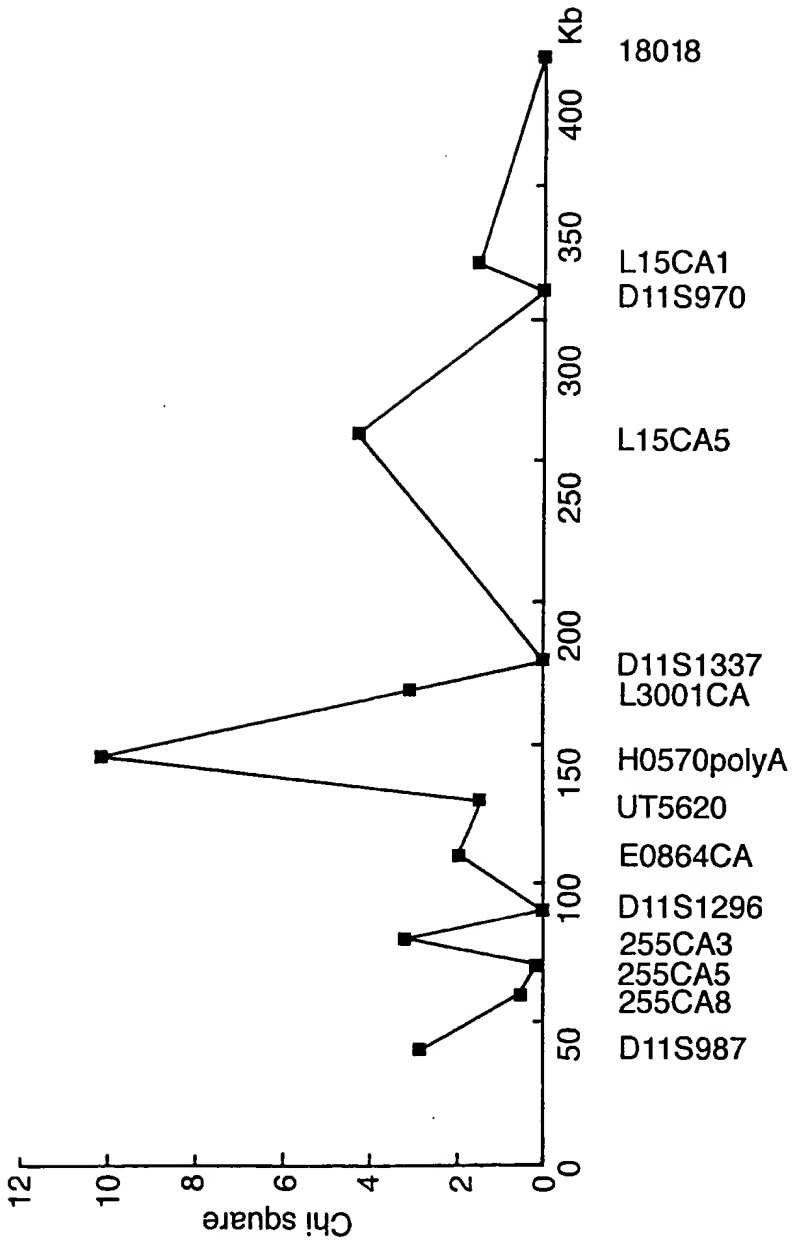
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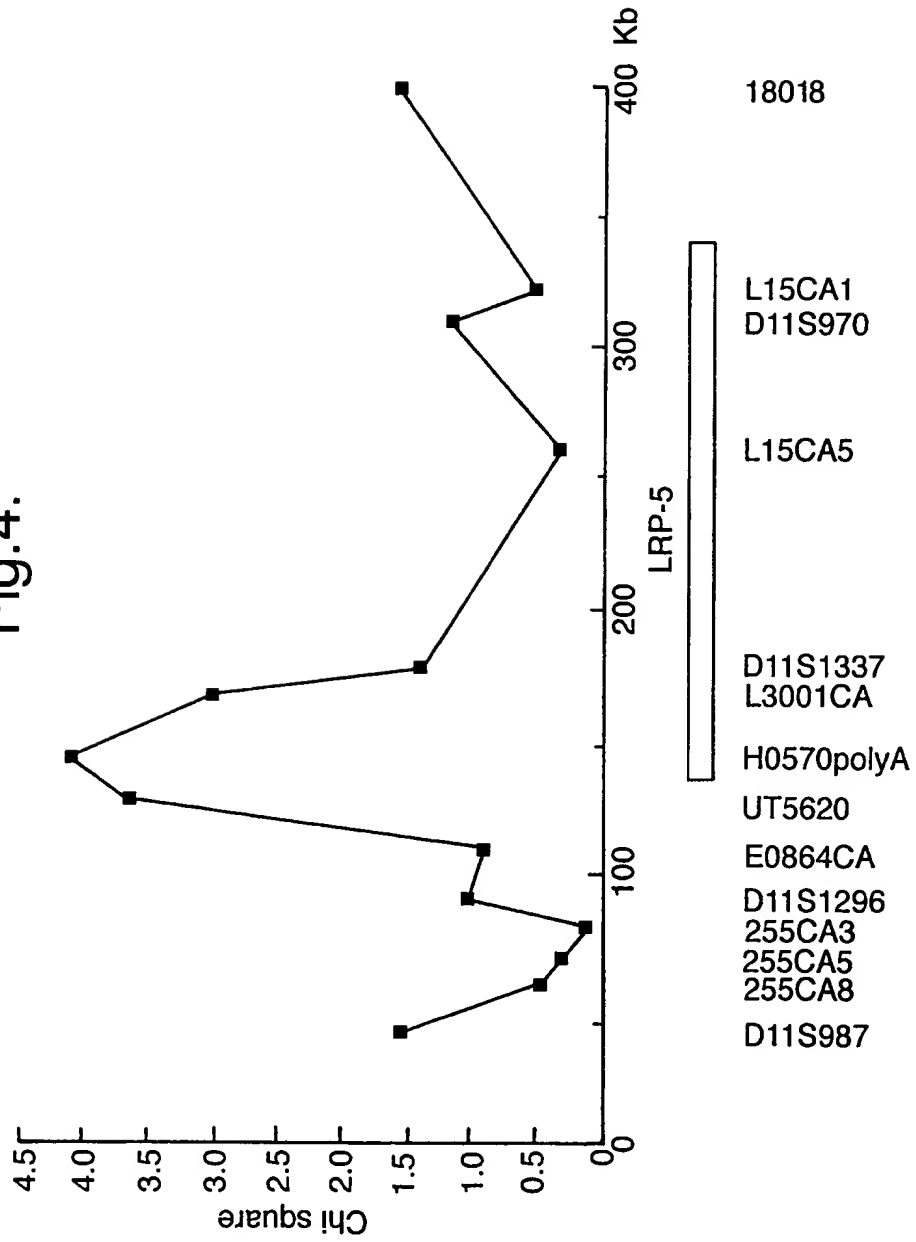
Fig.4.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400 Kb
LRP-5
18018
L15CA1
D11S970
L15CA5
D11S1337
L3001CA
H0570polyA
UT5620
E0864CA
D11S1296
255CA3
255CA5
255CA8
D11S987

Figure 5(a)

ATGGAGCCCGAGTGAGCGCGGCGCGGGCCCGTCCGGCCGCCGGACAACATGGAGG
CAGCGCCGCCCGGGCCGCCGTGGCCGCTGCTGCTGCTGCTGCTGCTGCTGGCG
CTGTGCGGCTGCCCGGCCCCCGCCGCGGCCTCGCCGCTCCTGCTATTTGCCAACCG
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GTCAGCGGCCTGGAGGATGCGGCCGCAGTGGACTTCCAGTTTTCCAAGGGAGCCGT
GTACTGGACAGACGTGAGCGAGGAGGCCATCAAGCAGACCTACCTGAACCAGACG
GGGGCCGCCGTGCAGAACGTGGTCATCTCCGGCCTGGTCTCTCCCGACGGCCTCGC
CTGCGACTGGGTGGGCAAGAAGCTGTACTGGACGGACTCAGAGACCAACCGCATC
GAGGTGGCCAACCTCAATGGCACATCCCGGAAGGTGCTCTTCTGGCAGGACCTTGA
CCAGCCGAGGGCCATCGCCTTGGACCCCGCTCACGGGTACATGTACTGGACAGACT
GGGGTGAGACGCCCCGGATTGAGCGGGCAGGGATGGATGGCAGCACCCGGAAGAT
CATTGTGGACTCGGACATTTACTGGCCCAATGGACTGACCATCGACCTGGAGGAGC
AGAAGCTCTACTGGGCTGACGCCAAGCTCAGCTTCATCCACCGTGCCAACCTGGAC
GGCTCGTTCCGGCAGAAGGTGGTGGAGGGCAGCCTGACGCACCCCTTCGCCCTGAC
GCTCTCCGGGGACACTCTGTACTGGACAGACTGGCAGACCCGCTCCATCCATGCCT
GCAACAAGCGCACTGGGGGGAAGAGGAAGGAGATCCTGAGTGCCCTCTACTCACC
CATGGACATCCAGGTGCTGAGCCAGGAGCGGCAGCCTTTCTTCCACACTCGCTGTG
AGGAGGACAATGGCGGCTGCTCCCACCTGTGCCTGCTGTCCCCAAGCGAGCCTTTC
TACACATGCGCCTGCCCCACGGGTGTGCAGCTGCAGGACAACGGCAGGACGTGTA
AGGCAGGAGCCGAGGAGGTGCTGCTGCTGGCCCGGCGGACGGACCTACGGAGGAT
CTCGCTGGACACGCCGGACTTTACCGACATCGTGCTGCAGGTGGACGACATCCGGC
ACGCCATTGCCATCGACTACGACCCGCTAGAGGGCTATGTCTACTGGACAGATGAC
GAGGTGCGGGCCATCCGCAGGGCGTACCTGGACGGGTCTGGGGCGCAGACGCTGG
TCAACACCGAGATCAACGACCCCGATGGCATCGCGGTCGACTGGGTGGCCCGAAA
CCTCTACTGGACCGACACGGGCACGGACCGCATCGAGGTGACGCGCCTCAACGGC
ACCTCCCGCAAGATCCTGGTGTCGGAGGACCTGGACGAGCCCCGAGCCATCGCACT
GCACCCCGTGATGGGCCTCATGTACTGGACAGACTGGGGAGAGAACCCTAAAATCG
AGTGTGCCAACTTGGATGGGCAGGAGCGGCGTGTGCTGGTCAATGCCTCCCTCGGG
TGGCCCAACGGCCTGGCCCTGGACCTGCAGGAGGGGAAGCTCTACTGGGGAGACG
CCAAGACAGACAAGATCGAGGTGATCAATGTTGATGGGACGAAGAGGCGGACCCT
CCTGGAGGACAAGCTCCCGCACATTTTCGGGTTCACGCTGCTGGGGGACTTCATCT
ACTGGACTGACTGGCAGCGCCGCAGCATCGAGCGGGTGCACAAGGTCAAGGCCAG
CCGGGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAAGCTGTGAATG
TGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACGGGGGGTGCAGCCA
CCTGTGCTTCTTCACACCCCACGCAACCCGGTGTGGCTGCCCCATCGGCCTGGAGC
TGCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCTTCTTGGTCTTCACCAGC
AGAGCCGCCATCCACAGGATCTCCCTCGAGACCAATAACAACGACGTGCCATCCCG
CTCACGGGCGTCAAGGAGGCCTCAGCCCTGGACTTTGATGTGTCCAACAACCACAT
CTACTGGACAGACGTCAGCCTGAAGACCATCAGCCGCGCCTTCATGAACGGGAGCT
CGGTGGAGCACGTGGTGGAGTTTGGCCTTGACTACCCCGAGGGCATGGCCGTTGAC
TGGATGGGCAAGAACCTCTACTGGGCCGACACTGGGACCAACAGAATCGAAGTGG
CGCGGCTGGACGGGCAGTTCCGGCAAGTCCTCGTGTGGAGGGACTTGGACAACCCG
AGGTCGCTGGCCCTGGATCCCACCAAGGGCTACATCTACTGGACCGAGTGGGGCGG

Figure 5(a) (Continued)

CAAGCCGAGGATCGTGCGGGCCTTCATGGACGGGACCAACTGCATGACGCTGGTGG
ACAAGGTGGGCCGGGCCAACGACCTCACCATTGACTACGCTGACCAGCGCCTCTAC
TGGACCGACCTGGACACCAACATGATCGAGTCGTCCAACATGCTGGGTCAGGAGCG
GGTCGTGATTGCCGACGATCTCCCGCACCCGTTCGGTCTGACGCAGTACAGCGATT
ATATCTACTGGACAGACTGGAATCTGCACAGCATTGAGCGGGCCGACAAGACTAGC
GGCCGGAACCGCACCCTCATCCAGGGCCACCTGGACTTCGTGATGGACATCCTGGT
GTTCCACTCCTCCCGCCAGGATGGCCTCAATGACTGTATGCACAACAACGGGCAGT
GTGGGCAGCTGTGCCTTGCCATCCCCGGCGGCCACCGCTGCGGCTGCGCCTCACAC
TACACCCTGGACCCCAGCAGCCGCAACTGCAGCCCGCCCACCACCTTCTTGCTGTT
CAGCCAGAAATCTGCCATCAGTCGGATGATCCCGGACGACCAGCACAGCCCGGATC
TCATCCTGCCCCTGCATGGACTGAGGAACGTCAAAGCCATCGACTATGACCCACTG
GACAAGTTCATCTACTGGGTGGATGGGCGCCAGAACATCAAGCGAGCCAAGGACG
ACGGGACCCAGCCCTTTGTTTTGACCTCTCTGAGCCAAGGCCAAAACCCAGACAGG
CAGCCCCACGACCTCAGCATCGACATCTACAGCCGGACACTGTTCTGGACGTGCGA
GGCCACCAATACCATCAACGTCCACAGGCTGAGCGGGGAAGCCATGGGGGTGGTG
CTGCGTGGGGACCGCGACAAGCCCAGGGCCATCGTCGTCAACGCGGAGCGAGGGT
ACCTGTACTTCACCAACATGCAGGACCGGGCAGCCAAGATCGAACGCGCAGCCCTG
GACGGCACCGAGCGCGAGGTCCTCTTCACCACCGGCCTCATCCGCCCTGTGGCCCT
GGTGGTAGACAACACACTGGGCAAGCTGTTCTGGGTGGACGCGGACCTGAAGCGC
ATTGAGAGCTGTGACCTGTCAGGGGCCAACCGCCTGACCCTGGAGGACGCCAACAT
CGTGCAGCCTCTGGGCCTGACCATCCTTGGCAAGCATCTCTACTGGATCGACCGCC
AGCAGCAGATGATCGAGCGTGTGGAGAAGACCACCGGGGACAAGCGGACTCGCAT
CCAGGGCCGTGTCGCCCACCTCACTGGCATCCATGCAGTGGAGGAAGTCAGCCTGG
AGGAGTTCTCAGCCCACCCATGTGCCCGTGACAATGGTGGCTGCTCCCACATCTGT
ATTGCCAAGGGTGATGGGACACCACGGTGCTCATGCCCAGTCCACCTCGTGCTCCT
GCAGAACCTGCTGACCTGTGGAGAGCCGCCCACCTGCTCCCCGGACCAGTTTGCAT
GTGCCACAGGGGAGATCGACTGTATCCCCGGGGCCTGGCGCTGTGACGGCTTTCCC
GAGTGCGATGACCAGAGCGACGAGGAGGGCTGCCCCGTGTGCTCCGCCGCCCAGTT
CCCCTGCGCGCGGGGTCAGTGTGTGGACCTGCGCCTGCGCTGCGACGGCGAGGCAG
ACTGTCAGGACCGCTCAGACGAGGCGGACTGTGACGCCATCTGCCTGCCCAACCAG
TTCCGGTGTGCGAGCGGCCAGTGTGTCCTATCAAACAGCAGTGCGACTCCTTCCCC
GACTGTATCGACGGCTCCGACGAGCTCATGTGTGAAATCACCAAGCCGCCCTCAGA
CGACAGCCCGGCCCACAGCAGTGCCATCGGGCCCGTCATTGGCATCATCCTCTCTC
TCTTCGTCATGGGTGGTGTCTATTTTGTGTGCCAGCGCGTGGTGTGCCAGCGCTATG
CGGGGGCCAACGGGCCCTTCCCGCACGAGTATGTCAGCGGGACCCCGCACGTGCCC
CTCAATTTCATAGCCCCGGGCGGTTCCCAGCATGGCCCCTTCACAGGCATCGCATG
CGGAAAGTCCATGATGAGCTCCGTGAGCCTGATGGGGGGCCGGGGCGGGGTGCCC
CTCTACGACCGGAACCACGTCACAGGGGCCTCGTCCAGCAGCTCGTCCAGCACGAA
GGCCACGCTGTACCCGCCGATCCTGAACCCGCCGCCCTCCCCGGCCACGGACCCCT
CCCTGTACAACATGGACATGTTCTACTCTTCAAACATTCCGGCCACTGTGAGACCG
TACAGGCCCTACATCATTCGAGGAATGGCGCCCCCGACGACGCCCTGCAGCACCGA
CGTGTGTGACAGCGACTACAGCGCCAGCCGCTGGAAGGCCAGCAAGTACTACCTG
GATTTGAACTCGGACTCAGACCCCTATCCACCCCCACCCACGCCCCACAGCCAGTA

Figure 5(a) (Continued)

CCTGTCGGCGGAGGACAGCTGCCCGCCCTCGCCCGCCACCGAGAGGAGCTACTTCC
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ACTCTGGCTTCTCTGTGCCCCTGTAAATAGTTTTAAATATGAACAAAGAAAAAAAT
ATATTTTATGATTTAAAAAATAAATATAATTGGGATTTTAAAAACATGAGAAATGT
GAACTGTGATGGGGTGGGCAGGGCTGGGAGAACTTTGTACAGTGGAACAAATATTT
ATAAACTTAATTTTGTAAAACAG

Figure 5(b)

ATGGAGGCAGCGCCGCCCGGGCCGCCGTGGCCGCTGCTGCTGCTGCTGCTGCTG
CTGCTGGCGCTGTGCGGCTGCCCGGCCCCCGCCGCGGCCTCGCCGCTCCTGCTA
TTTGCCAACCGCCGGGACGTACGGCTGGTGGACGCCGGCGGAGTCAAGCTGGA
GTCCACCATCGTGGTCAGCGGCCTGGAGGATGCGGCCGCAGTGGACTTCCAGTT
TTCCAAGGGAGCCGTGTACTGGACAGACGTGAGCGAGGAGGCCATCAAGCAGA
CCTACCTGAACCAGACGGGGGCCGCCGTGCAGAACGTGGTCATCTCCGGCCTGG
TCTCTCCCGACGGCCTCGCCTGCGACTGGGTGGGCAAGAAGCTGTACTGGACGG
ACTCAGAGACCAACCGCATCGAGGTGGCCAACCTCAATGGCACATCCCGGAAG
GTGCTCTTCTGGCAGGACCTTGACCAGCCGAGGGCCATCGCCTTGGACCCCGCT
CACGGGTACATGTACTGGACAGACTGGGGTGAGACGCCCCGGATTGAGCGGGC
AGGGATGGATGGCAGCACCCGGAAGATCATTGTGGACTCGGACATTTACTGGCC
CAATGGACTGACCATCGACCTGGAGGAGCAGAAGCTCTACTGGGCTGACGCCA
AGCTCAGCTTCATCCACCGTGCCAACCTGGACGGCTCGTTCCGGCAGAAGGTGG
TGGAGGGCAGCCTGACGCACCCCTTCGCCCTGACGCTCTCCGGGGACACTCTGT
ACTGGACAGACTGGCAGACCCGCTCCATCCATGCCTGCAACAAGCGCACTGGGG
GGAAGAGGAAGGAGATCCTGAGTGCCCTCTACTCACCCATGGACATCCAGGTGC
TGAGCCAGGAGCGGCAGCCTTTCTTCCACACTCGCTGTGAGGAGGACAATGGCG
GCTGCTCCCACCTGTGCCTGCTGTCCCCAAGCGAGCCTTTCTACACATGCGCCT
GCCCCACGGGTGTGCAGCTGCAGGACAACGGCAGGACGTGTAAGGCAGGAGCC
GAGGAGGTGCTGCTGCTGGCCCGGCGGACGGACCTACGGAGGATCTCGCTGGA
CACGCCGGACTTTACCGACATCGTGCTGCAGGTGGACGACATCCGGCACGCCAT
TGCCATCGACTACGACCCGCTAGAGGGCTATGTCTACTGGACAGATGACGAGGT
GCGGGCCATCCGCAGGGCGTACCTGGACGGGTCTGGGGCGCAGACGCTGGTCA
ACACCGAGATCAACGACCCCGATGGCATCGCGGTCGACTGGGTGGCCCGAAAC
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CACCTCCCGCAAGATCCTGGTGTCGGAGGACCTGGACGAGCCCCGAGCCATCGC
ACTGCACCCCGTGATGGGCCTCATGTACTGGACAGACTGGGGAGAGAACCCTAA
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CCTCGGGTGGCCCAACGGCCTGGCCCTGGACCTGCAGGAGGGGAAGCTCTACTG
GGGAGACGCCAAGACAGACAAGATCGAGGTGATCAATGTTGATGGGACGAAGA
GGCGGACCCTCCTGGAGGACAAGCTCCCGCACATTTTCGGGTTCACGCTGCTGG
GGGACTTCATCTACTGGACTGACTGGCAGCGCCGCAGCATCGAGCGGGTGCACA
AGGTCAAGGCCAGCCGGGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGC
TCAAAGCTGTGAATGTGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGG
AACGGGGGGTGCAGCCACCTGTGCTTCTTCACACCCCACGCAACCCGGTGTGGC
TGCCCCATCGGCCTGGAGCTGCTGAGTGACATGAAGACCTGCATCGTGCCTGAG
GCCTTCTTGGTCTTCACCAGCAGAGCCGCCATCCACAGGATCTCCCTCGAGACC
AATAACAACGACGTGGCCATCCCGCTCACGGGCGTCAAGGAGGCCTCAGCCCTG
GACTTTGAGTGTCCAACAACCACATCTACTGGACAGACGTCAGCCTGAAGACCA
TCAGCCGCGCCTTCATGAACGGGAGCTCGGTGGAGCACGTGGTGGAGTTTGGCC
TTGACTACCCCGAGGGCATGGCCGTTGACTGGATGGGCAAGAACCTCTACTGGG
CCGACACTGGGACCAACAGAATCGAAGTGGCGCGGCTGGACGGGCAGTTCCGG
CAAGTCCTCGTGTGGAGGGACTTGGACAACCCGAGGTCGCTGGCCCTGGATCCC
ACCAAGGGCTACATCTACTGGACCGAGTGGGGCGGCAAGCCGAGGATCGTGCG
GGCCTTCATGGACGGGACCAACTGCATGACGCTGGTGGACAAGGTGGGCCGGG
CCAACGACCTCACCATTGACTACGCTGACCAGCGCCTCTACTGGACCGACCTGG

Figure 5(b) (Continued)

ACACCAACATGATCGAGTCGTCCAACATGCTGGGTCAGGAGCGGGTCGTGATTG
CCGACGATCTCCCGCACCCGTTCGGTCTGACGCAGTACAGCGATTATATCTACT
GGACAGACTGGAATCTGCACAGCATTGAGCGGGCCGACAAGACTAGCGGCCGG
AACCGCACCCTCATCCAGGGCCACCTGGACTTCGTGATGGACATCCTGGTGTTC
CACTCCTCCCGCCAGGATGGCCTCAATGACTGTATGCACAACAACGGGCAGTGT
GGGCAGCTGTGCCTTGCCATCCCCGGCGGCCACCGCTGCGGCTGCGCCTCACAC
TACACCCTGGACCCCAGCAGCCGCAACTGCAGCCCGCCCACCACCTTCTTGCTG
TTCAGCCAGAAATCTGCCATCAGTCGGATGATCCCGGACGACCAGCACAGCCCG
GATCTCATCCTGCCCCTGCATGGACTGAGGAACGTCAAAGCCATCGACTATGAC
CCACTGGACAAGTTCATCTACTGGGTGGATGGGCGCCAGAACATCAAGCGAGCC
AAGGACGACGGGACCCAGCCCTTTGTTTTGACCTCTCTGAGCCAAGGCCAAAAC
CCAGACAGGCAGCCCCACGACCTCAGCATCGACATCTACAGCCGGACACTGTTC
TGGACGTGCGAGGCCACCAATACCATCAACGTCCACAGGCTGAGCGGGGAAGC
CATGGGGGTGGTGCTGCGTGGGGACCGCGACAAGCCCAGGGCCATCGTCGTCA
ACGCGGAGCGAGGGTACCTGTACTTCACCAACATGCAGGACCGGGCAGCCAAG
ATCGAACGCGCAGCCCTGGACGGCACCGAGCGCGAGGTCCTCTTCACCACCGGC
CTCATCCGCCCTGTGGCCCTGGTGGTAGACAACACACTGGGCAAGCTGTTCTGG
GTGGACGCGGACCTGAAGCGCATTGAGAGCTGTGACCTGTCAGGGGCCAACCG
CCTGACCCTGGAGGACGCCAACATCGTGCAGCCTCTGGGCCTGACCATCCTTGG
CAAGCATCTCTACTGGATCGACCGCCAGCAGCAGATGATCGAGCGTGTGGAGAA
GACCACCGGGGACAAGCGGACTCGCATCCAGGGCCGTGTCGCCCACCTCACTGG
CATCCATGCAGTGGAGGAAGTCAGCCTGGAGGAGTTCTCAGCCCACCCATGTGC
CCGTGACAATGGTGGCTGCTCCCACATCTGTATTGCCAAGGGTGATGGGACACC
ACGGTGCTCATGCCCAGTCCACCTCGTGCTCCTGCAGAACCTGCTGACCTGTGG
AGAGCCGCCCACCTGCTCCCCGGACCAGTTTGCATGTGCCACAGGGGAGATCGA
CTGTATCCCCGGGGCCTGGCGCTGTGACGGCTTTCCCGAGTGCGATGACCAGAG
CGACGAGGAGGGCTGCCCCGTGTGCTCCGCCGCCCAGTTCCCCTGCGCGCGGGG
TCAGTGTGTGGACCTGCGCCTGCGCTGCGACGGCGAGGCAGACTGTCAGGACCG
CTCAGACGAGGCGGACTGTGACGCCATCTGCCTGCCCAACCAGTTCCGGTGTGC
GAGCGGCCAGTGTGTCCTCATCAAACAGCAGTGCGACTCCTTCCCCGACTGTAT
CGACGGCTCCGAGAGCTCATGTGTGAAATCACCAAGCCGCCCTCAGACGACAGC
CCGGCCCACAGCAGTGCCATCGGGCCCGTCATTGGCATCATCCTCTCTCTCTTC
GTCATGGGTGGTGTCTATTTTGTGTGCCAGCGCGTGGTGTGCCAGCGCTATGCG
GGGGCCAACGGGCCCTTCCCGCACGAGTATGTCAGCGGGACCCCGCACGTGCCC
CTCAATTTCATAGCCCCGGGCGGTTCCCAGCATGGCCCCTTCACAGGCATCGCA
TGCGGAAAGTCCATGATGAGCTCCGTGAGCCTGATGGGGGGCCGGGGCGGGGT
GCCCCTCTACGACCGGAACCACGTCACAGGGGCCTCGTCCAGCAGCTCGTCCAG
CACGAAGGCCACGCTGTACCCGCCGATCCTGAACCCGCCGCCCTCCCCGGCCAC
GGACCCCTCCCTGTACAACATGGACATGTTCTACTCTTCAAACATTCCGGCCAC
TGTGAGACCGTACAGGCCCTACATCATTCGAGGAATGGCGCCCCCGACGACGCC
CTGCAGCACCGACGTGTGTGACAGCGACTACAGCGCCAGCCGCTGGAAGGCCA
GCAAGTACTACCTGGATTTGAACTCGGACTCAGACCCCTATCCACCCCCACCCA
CGCCCCACAGCCAGTACCTGTCGGCGGAGGACAGCTGCCCGCCCTCGCCCGCCA
CCGAGAGGAGCTACTTCCATCTCTTCCCGCCCCCTCCGTCCCCCTGCACGGA
CTCATCC

Figure 5(c)

MEAAPPGPPWPLLLLLLLLLALCGCPAPAAASPLLLFANRRDVRLVDAGGVKLESTIV
VSGLEDAAAVDFQFSKGAVYWTDVSEEAIKQTYLNQTGAAVQNVVISGLVSPDGLAC
DWVGKKLYWTDSETNRIEVANLNGTSRKVLFWQDLDQPRAIALDPAHGYMYWTDW
GETPRIERAGMDGSTRKIIVDSDIYWPNGLTIDLEEQKLYWADAKLSFIHRANLDGSFR
QKVVEGSLTHPFALTLSGDTLYWTDWQTRSIHACNKRTGGKRKEILSALYSPMDIQVLS
QERQPFFHTRCEEDNGGCSHLCLLSPSEPFYTCACPTGVQLQDNGRTCKAGAEEVLLL
ARRTDLRRISLDTPDFTDIVLQVDDIRHAIAIDYDPLEGYVYWTDDEVRAIRRAYLDGS
GAQTLVNTEINDPDGIAVDWVARNLYWTDTGTDRIEVTRLNGTSRKILVSEDLDEPRAI
ALHPVMGLMYWTDWGENPKIECANLDGQERRVLVNASLGWPNGLALDLQEGKLYW
GDAKTDKIEVINVDGTKRRTLLEDKLPHIFGFTLLGDFIYWTDWQRRSIERVHKVKASR
DVIIDQLPDLMGLKAVNVAKVVGTNPCADRNGGCSHLCFFTPHATRCGCPIGLELLSD
MKTCIVPEAFLVFTSRAAIHRISLETNNNDVAIPLTGVKEASALDFDVSNNHIYWTDVSL
KTISRAFMNGSSVEHVVEFGLDYPEGMAVDWMGKNLYWADTGTNRIEVARLDGQFR
QVLVWRDLDNPRSLALDPTKGYIYWTEWGGKPRIVRAFMDGTNCMTLVDKVGRAND
LTIDYADQRLYWTDLDTNMIESSNMLGQERVVIADDLPHPFGLTQYSDYIYWTDWNL
HSIERADKTSGRNRTLIQGHLDFVMDILVFHSSRQDGLNDCMHNNGQCGQLCLAIPGG
HRCGCASHYTLDPSSRNCSPPTTFLLFSQKSAISRMIPDDQHSPDLILPLHGLRNVKAIDY
DPLDKFIYWVDGRQNIKRAKDDGTQPFVLTSLSQGQNPDRQPHDLSIDIYSRTLFWTCE
ATNTINVHRLSGEAMGVVLRGDRDKPRAIVVNAERGYLYFTNMQDRAAKIERAALDG
TEREVLFTTGLIRPVALVVDNTLGKLFWVDADLKRIESCDLSGANRLTLEDANIVQPLG
LTILGKHLYWIDRQQQMIERVEKTTGDKRTRIQGRVAHLTGIHAVEEVSLEEFSAHPCA
RDNGGCSHICIAKGDGTPRCSCPVHLVLLQNLLTCGEPPTCSPDQFACATGEIDCIPGA
WRCDGFPECDDQSDEEGCPVCSAAQFPCARGQCVDLRLRCDGEADCQDRSDEADCD
AICLPNQFRCASGQCVLIKQQCDSFPDCIDGSDELMCEITKPPSDDSPAHSSAIGPVIGIIL
SLFVMGGVYFVCQRVVCQRYAGANGPFPHEYVSGTPHVPLNFIAPGGSQHGPFTGIAC
GKSMMSSVSLMGGRGGVPLYDRNHVTGASSSSSSSTKATLYPPILNPPPSPATDPSLYN
MDMFYSSNIPATVRPYRPYIIRGMAPPTTPCSTDVCDSDYSASRWKASKYYLDLNSDSD
PYPPPPTPHSQYLSAEDSCPPSPATERSYFHLFPPPPSPCTDSS

Figure 5(d)

```
MEAAPPGPPW PLLLLLLLLL ALCGCPAPAA ASPLLLFANR RDVRLVDAGG    50
VKLESTIVVS GLEDAAAVDF QFSKGAVYWT DVSEEAIKQT YLNQTGAAVQ   100
NVVISGLVSP DGLACDWVGK KLYWTDSETN RIEVANLNGT SRKVLFWQDL   150
DQPRAIALDP AHGYMYWTDW GETPRIERAG MDGSTRKIIV DSDIYWPNGL   200
TIDLEEQKLY WADAKLSFIH RANLDGSFRQ KVVEGSLTHP FALTLSGDTL   250
YWTDWQTRSI HACNKRTGGK RKEILSALYS PMDIQVLSQE RQPFFHTRCE   300
EDNGGCSHLC LLSPSEPFYT CACPTGVQLQ DNGRTCKAGA EEVLLLARRT   350
DLRRISLDTP DFTDIVLQVD DIRHAIAIDY DPLEGYVYWT DDEVRAIRRA   400
YLDGSGAQTL VNTEINDPDG IAVDWVARNL YWTDTGTDRI EVTRLNGTSR   450
KILVSEDLDE PRAIALHPVM GLMYWTDWGE NPKIECANLD GQERRVLVNA   500
SLGWPNGLAL DLQEGKLYWG DAKTDKIEVI NVDGTKRRTL LEDKLPHIFG   550
FTLLGDFIYW TDWQRRSIER VHKVKASRDV IIDQLPDLMG LKAVNVAKVV   600
GTNPCADRNG GCSHLCFFTP HATRCGCPIG LELLSDMKTC IVPEAFLVFT   650
SRAAIHRISL ETNNNDVAIP LTGVKEASAL DFDVSNNHIY WTDVSLKTIS   700
RAFMNGSSVE HVVEFGLDYP EGMAVDWMGK NLYWADTGTN RIEVARLDGQ   750
FRQVLVWRDL DNPRSLALDP TKGYIYWTEW GGKPRIVRAF MDGTNCMTLV   800
DKVGRANDLT IDYADQRLYW TDLDTNMIES SNMLGQERVV IADDLPHPFG   850
LTQYSDYIYW TDWNLHSIER ADKTSGRNRT LIQGHLDFVM DILVFHSSRQ   900
DGLNDCMHNN GQCGQLCLAI PGGHRCGCAS HYTLDPSSRN CSPPTTFLLF   950
SQKSAISRMI PDDQHSPDLI LPLHGLRNVK AIDYDPLDKF IYWVDGRQNI  1000
KRAKDDGTQP FVLTSLSQGQ NPDRQPHDLS IDIYSRTLFW TCEATNTINV  1050
HRLSGEAMGV VLRGDRDKPR AIVVNAERGY LYFTNMQDRA AKIERAALDG  1100
TEREVLFTTG LIRPVALVVD NTLGKLFWVD ADLKRIESCD LSGANRLTLE  1150
DANIVQPLGL TILGKHLYWI DRQQQMIERV EKTTGDKRTR IQGRVAHLTG  1200
IHAVEEVSLE EFSAHPCARD NGGCSHICIA KGDGTPRCSC PVHLVLLQNL  1250
LTCGEPPTCS PDQFACATGE IDCIPGAWRC DGFPECDDQS DEEGCPVCSA  1300
AQFPCARGQC VDLRLRCDGE ADCQDRSDEA DCDAICLPNQ FRCASGQCVL  1350
IKQQCDSFPD CIDGSDELMC EITKPPSDDS PAHSSAIGPV IGIILSLFVM  1400
GGVYFVCQRV VCQRYAGANG PFPHEYVSGT PHVPLNFIAP GGSQHGPFTG  1450
IACGKSMMSS VSLMGGRGGV PLYDRNHVTG ASSSSSSSTK ATLYPPILNP  1500
PPSPATDPSL YNMDMFYSSN IPATVRPYRP YIIRGMAPPT TPCSTDVCDS  1550
DYSASRWKAS KYYLDLNSDS DPYPPPPTPH SQYLSAEDSC PPSPATERSY  1600
FHLFPPPPSP CTDSS                                       1615
```

Figure 5(e)

CPAPAAASPLLLFANRRDVRLVDAGGVKLESTIVVSGLEDAAAVDFQFSKGAVYWTD
VSEEAIKQTYLNQTGAAVQNVVISGLVSPDGLACDWVGKKLYWTDSETNRIEVANLN
GTSRKVLFWQDLDQPRAIALDPAHGYMYWTDWGETPRIERAGMDGSTRKIIVDSDIY
WPNGLTIDLEEQKLYWADAKLSFIHRANLDGSFRQKVVEGSLTHPFALTLSGDTLYWT
DWQTRSIHACNKRTGGKRKEILSALYSPMDIQVLSQERQPFFHTRCEEDNGGCSHLCLL
SPSEPFYTCACPTGVQLQDNGRTCKAGAEEVLLLARRTDLRRISLDTPDFTDIVLQVDDI
RHAIAIDYDPLEGYVYWTDDEVRAIRRAYLDGSGAQTLVNTEINDPDGIAVDWVARNL
YWTDTGTDRIEVTRLNGTSRKILVSEDLDEPRAIALHPVMGLMYWTDWGENPKIECAN
LDGQERRVLVNASLGWPNGLALDLQEGKLYWGDAKTDKIEVINVDGTKRRTLLEDKL
PHIFGFTLLGDFIYWTDWQRRSIERVHKVKASRDVIIDQLPDLMGLKAVNVAKVVGTN
PCADRNGGCSHLCFFTPHATRCGCPIGLELLSDMKTCIVPEAFLVFTSRAAIHRISLETN
NNDVAIPLTGVKEASALDFDVSNNHIYWTDVSLKTISRAFMNGSSVEHVVEFGLDYPE
GMAVDWMGKNLYWADTGTNRIEVARLDGQFRQVLVWRDLDNPRSLALDPTKGYIY
WTEWGGKPRIVRAFMDGTNCMTLVDKVGRANDLTIDYADQRLYWTDLDTNMIESSN
MLGQERVVIADDLPHPFGLTQYSDYIYWTDWNLHSIERADKTSGRNRTLIQGHLDFVM
DILVFHSSRQDGLNDCMHNNGQCGQLCLAIPGGHRCGCASHYTLDPSSRNCSPPTTFLL
FSQKSAISRMIPDDQHSPDLILPLHGLRNVKAIDYDPLDKFIYWVDGRQNIKRAKDDGT
QPFVLTSLSQGQNPDRQPHDLSIDIYSRTLFWTCEATNTINVHRLSGEAMGVVLRGDRD
KPRAIVVNAERGYLYFTNMQDRAAKIERAALDGTEREVLFTTGLIRPVALVVDNTLGK
LFWVDADLKRIESCDLSGANRLTLEDANIVQPLGLTILGKHLYWIDRQQQMIERVEKTT
GDKRTRIQGRVAHLTGIHAVEEVSLEEFSAHPCARDNGGCSHICIAKGDGTPRCSCPVH
LVLLQNLLTCGEPPTCSPDQFACATGEIDCIPGAWRCDGFPECDDQSDEEGCPVCSAAQ
FPCARGQCVDLRLRCDGEADCQDRSDEADCDAICLPNQFRCASGQCVLIKQQCDSFPD
CIDGSDELMCEITKPPSDDSPAHSSAIGPVIGIILSLFVMGGVYFVCQRVVCQRYAGANG
PFPHEYVSGTPHVPLNFIAPGGSQHGPFTGIACGKSMMSSVSLMGGRGGVPLYDRNHV
TGASSSSSSSTKATLYPPILNPPPSPATDPSLYNMDMFYSSNIPATVRPYRPYIIRGMAPPT
TPCSTDVCDSDYSASRWKASKYYLDLNSDSDPYPPPPTPHSQYLSAEDSCPPSPATERSY
FHLFPPPPSPCTDSS

Figure 5f

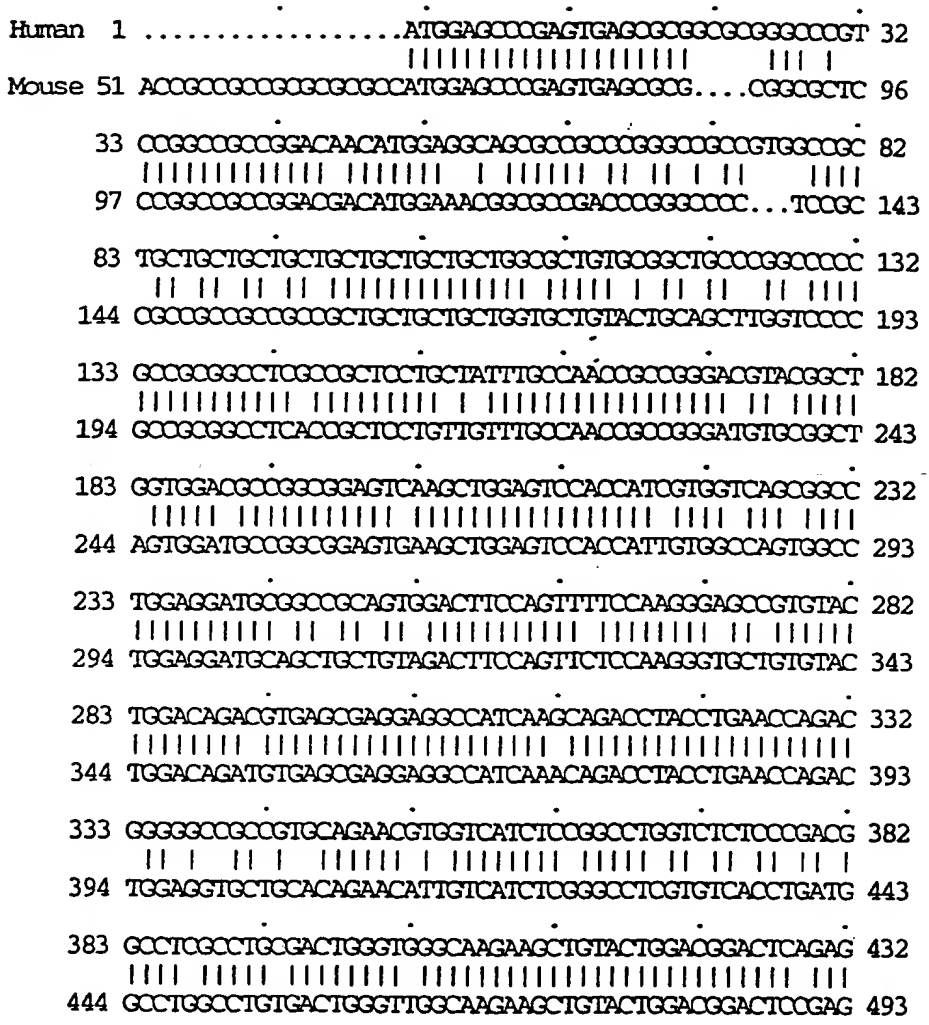
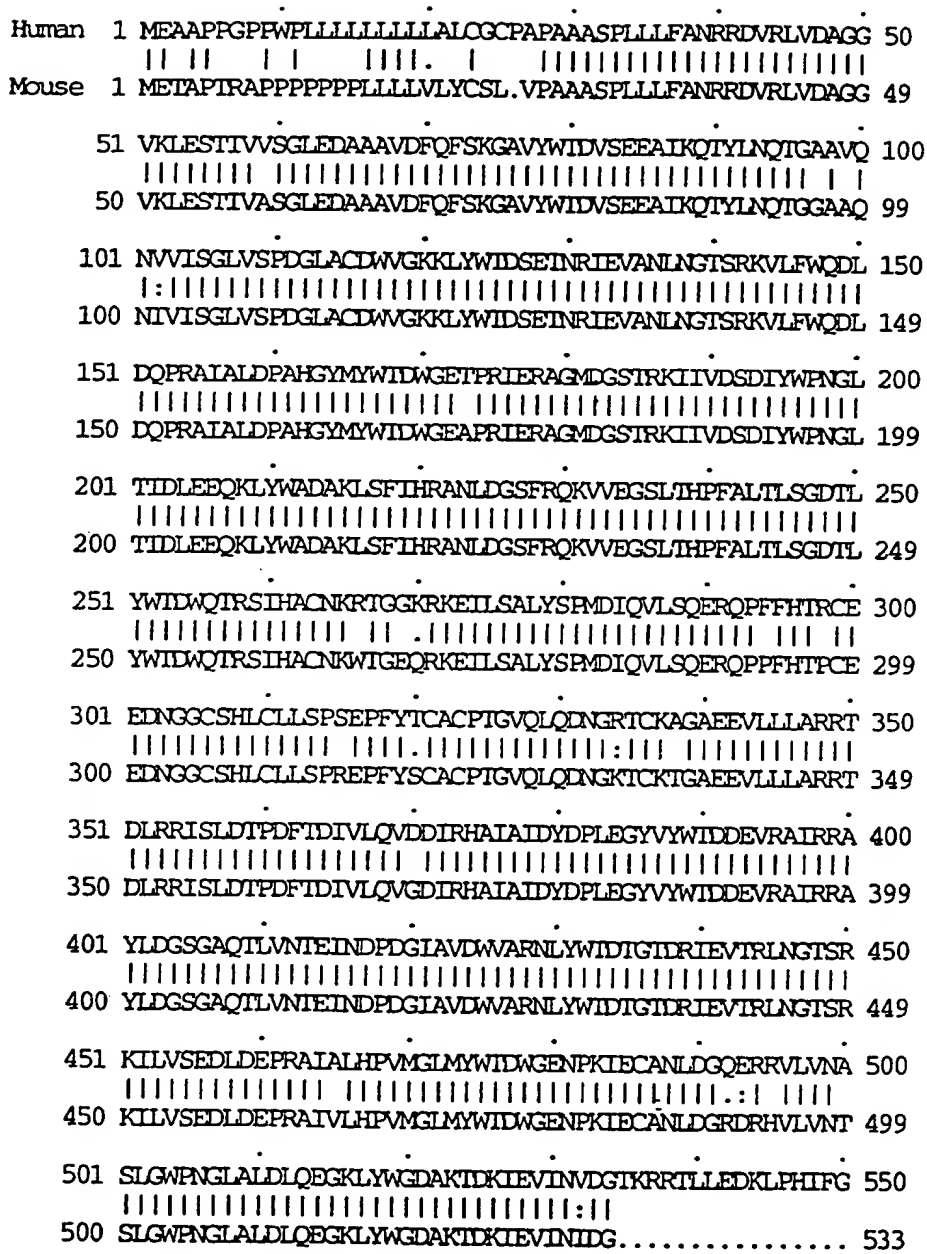

Human 1 ATGGAGCCCGAGTGAGCGCGGCGCGGGCCCGT 32
Mouse 51 ACCGCCGCCCGCGCGCGCCATGGAGCCCGAGTGAGCGCG....CGGCGCTC 96
33 CCGGCCGCCGGACAACATGGAGGCAGCGCCGCCCGGGCGCCGTGGCCGC 82
97 CCGGCCGCCGGACGACATGGAAACGGCGCCGACCCGGCCCC...TCCGC 143
83 TGCTGCTGCTGCTGCTGCTGCTGGCGCTGTGCGGCTGCCCGGCCCCC 132
144 CGCCGCCGCCGCCGCTGCTGCTGCTGGTGCTGTACTGCAGCTTGGTCCCC 193
133 GCCGCGGCCTCGCCGCTCCTGCTATTTGCCAACCGCCGGGACGTACGGCT 182
194 GCCGCGGCCTCACCGCTCCTGTTGTTTGCCAACCGCCGGGATGTGCGGCT 243
183 GGTGGACGCCGGCGGAGTCAAGCTGGAGTCCACCATCGTGGTCAGCGGCC 232
244 AGTGGATGCCGGCGGAGTGAAGCTGGAGTCCACCATTGTGGCCAGTGGCC 293
233 TGGAGGATGCGGCCGCAGTGGACTTCCAGTTTTCCAAGGGAGCCGTGTAC 282
294 TGGAGGATGCAGCTGCTGTAGACTTCCAGTTCTCCAAGGGTGCTGTGTAC 343
283 TGGACAGACGTGAGCGAGGAGGCCATCAAGCAGACCTACCTGAACCAGAC 332
344 TGGACAGATGTGAGCGAGGAGGCCATCAAACAGACCTACCTGAACCAGAC 393
333 GGGGGCCGCCGTGCAGAACGTGGTCATCTCCGGCCTGGTCTCTCCCGACG 382
394 TGGAGGTGCTGCACAGAACATTGTCATCTCGGGCCTCGTGTCACCTGATG 443
383 GCCTCGCCTGCGACTGGGTGGGCAAGAAGCTGTACTGGACGGACTCAGAG 432
444 GCCTGGCCTGTGACTGGGTTGGCAAGAAGCTGTACTGGACGGACTCCGAG 493

Figure 5g


Human 1 MEAAPPGPPWPLLLLLLLLLALCGCPAPAAASPLLLFANRRDVRLVDAGG 50
Mouse 1 METAPTRAPPPPPPPLLLLVLYCSL.VPAAASPLLLFANRRDVRLVDAGG 49
51 VKLESTIVVSGLEDAAAVDFQFSKGAVYWIDVSEEAIKQTYLNQTGAAVQ 100
50 VKLESTIVASGLEDAAAVDFQFSKGAVYWIDVSEEAIKQTYLNQTGGAAQ 99
101 NVVISGLVSPDGLACDWVGKKLYWIDSEINRIEVANLNGTSRKVLFWQDL 150
100 NIVISGLVSPDGLACDWVGKKLYWIDSEINRIEVANLNGTSRKVLFWQDL 149
151 DQPRAIALDPAHGYMYWIDWGETPRIERAGMDGSTRKIIVDSDIYWPNGL 200
150 DQPRAIALDPAHGYMYWIDWGEAPRIERAGMDGSTRKIIVDSDIYWPNGL 199
201 TIDLEEQKLYWADAKLSFIHRANLDGSFRQKVVEGSLTHPFALTLSGDTL 250
200 TIDLEEQKLYWADAKLSFIHRANLDGSFRQKVVEGSLTHPFALTLSGDTL 249
251 YWIDWQTRSIHACNKRTGGKRKEILSALYSPMDIQVLSQERQPFFHTRCE 300
250 YWIDWQTRSIHACNKWTGEQRKEILSALYSPMDIQVLSQERQPPFHTPCE 299
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300 EDNGGCSHLCLLSPREPFYSCACPTGVQLQDNGKTCKTGAEEVLLLARRT 349
351 DLRRISLDTPDFTDIVLQVDDIRHAIAIDYDPLEGYVYWIDDEVRAIRRA 400
350 DLRRISLDTPDFTDIVLQVGDIRHAIAIDYDPLEGYVYWIDDEVRAIRRA 399
401 YLDGSGAQTLVNTEINDPDGIAVDWVARNLYWIDTGTDRIEVTRLNGTSR 450
400 YLDGSGAQTLVNTEINDPDGIAVDWVARNLYWIDTGTDRIEVTRLNGTSR 449
451 KILVSEDLDEPRAIALHPVMGLMYWIDWGENPKIECANLDGQERRVLVNA 500
450 KILVSEDLDEPRAIVLHPVMGLMYWIDWGENPKIECANLDGRDRHVLVNT 499
501 SLGWPNGLALDLQEGKLYWGDAKTDKIEVINVDGTKRRILLEDKLPHIFG 550
500 SLGWPNGLALDLQEGKLYWGDAKTDKIEVINIDG..... 533

Figure 6(a)

EGF precursor motifs in LRP-5 isoform 1

```
Isoform 1     268   CEEDNGGCSHLCLLSPSEPFYTCACPTGVQLQDNGRTC 345
                    C   NGGCS LCLLSP  -  CACPT   L   GRTC
LRP-EGF repeat      CKVNNGGCSNLCLLSPGGG-HKCACPINFYLGSDGRTC

Isoform 1     570 GINPCADRNGGCSHLCFFTPHATRCGCPIGLELLSDMKTCI 650
                  GIN C   NGGCS LC  TP    C C     L  D  TC
LRP-EGF repeat    GINKCRVNNGGCSSLCLATPGSRQCACAEDQVLDADGVTCL

Isoform 1     871 GLNDCMHNNGQCGQLCLAIPGGHRCGCASHYTLDPSSRNC 950
                  G N C  NNG C  LCLA PG   C CA    LD     C
LRP-EGF repeat    GINKCRVNNGGCSSLCLATPGSRQCACAEDQVLDADGVTC

Isoform 1    1184  HPCARDNGGCSHICIAKGDGTPRCSCPVHLVLLQNLLTC 1262
                   HPC   NGGCS  C     G   C CP    L     TC
LRP-EGF repeat     HPCKVNNGGCSNLCLLSPGGGHKCACPINFYLGSDGRTC
```

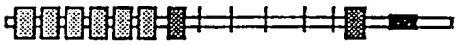
LDL-receptor motifs in LRP-5 isoform 1

```
Isoform 1    1226  PTCSPDQFACATGEIDCIPGAWRCDGFPECDDQSDEEGC 1304
                   P C  DQF C  G --CIP  WRCD    C D SDEE C
LRP-LDL repeat     PRCDMDQFQCKSGH--CIPLRWRCDADADCMDGSDEEAC

Isoform 1    1267    CSAAQFPCARGQCVDLRLRCDGEADCQDRSDEADCD 1342
                     C   QF C  G C      CDG   DCQD SDEA CD
LRP-LDL repeat       CRPGQFQCSTGICINPAFICDGDNDCQDNSDEANCD

Isoform 1    1305     CLPNQFRCASGQCVLIKQQCDSFPDCIDGSDELMC 1379
                     C   QF C SG C      CD   DC DGSDE  C
LRP-LDL repeat       CDMDQFQCKSGHCIPLRWRCDADADCMDGSDEEAC 37
```

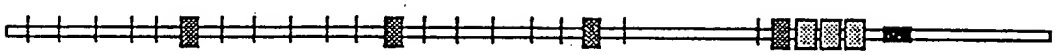
Figure 6(b)

Motif Organization of the LDL- receptor and LRP-5

LDL -receptor



LRP-5



EGF-precursor B.2 motif

LDL-receptor motif

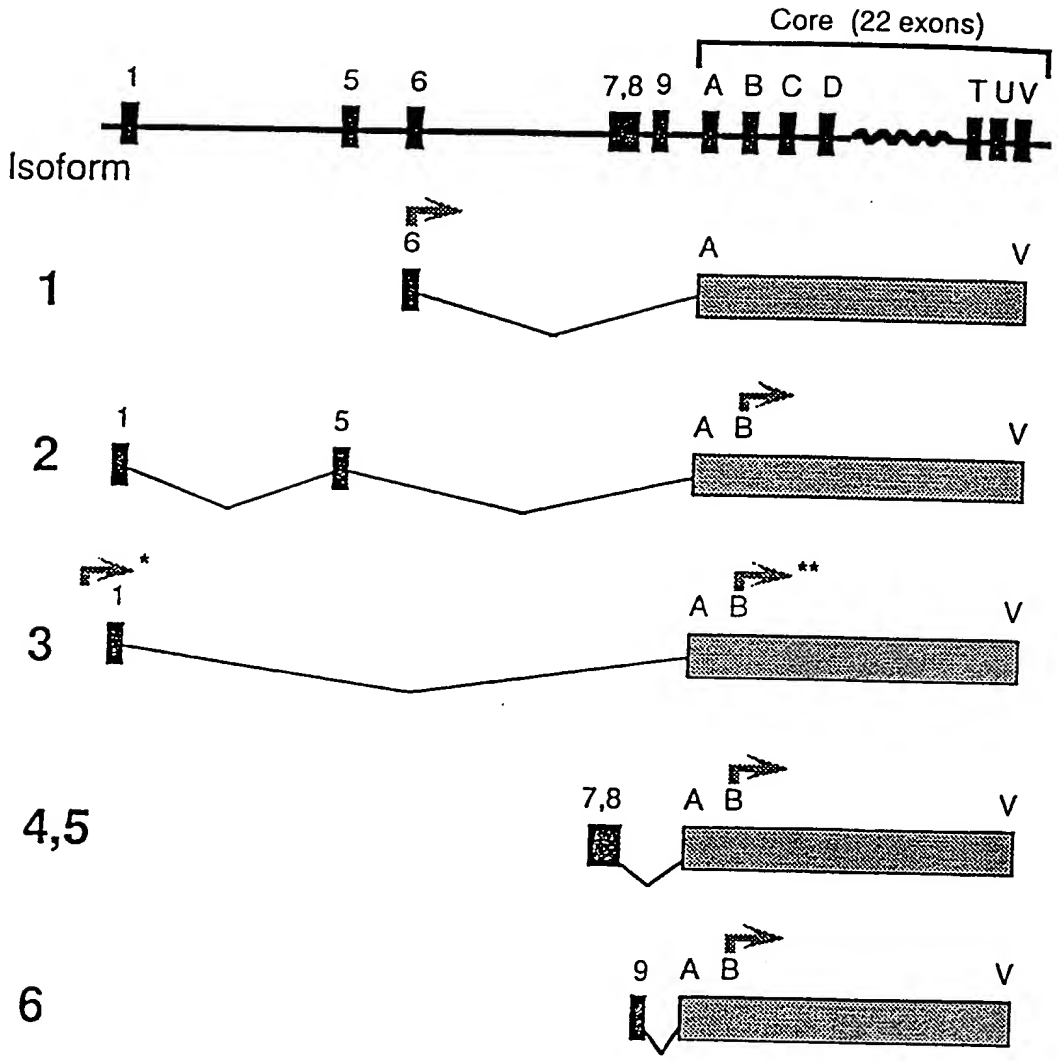
YWTD motif

Transmembrane region


D11S970
D11S1337
L3001 CA
H0570Poly A
D11S1917/UT 5620
1
5 6
7,8 9
A B C
D
E F
G H
I J K L M N O P
Q R S T U V
10kb
Genomic Sequence
Exon (not to scale)

Figure 7

Figure 8



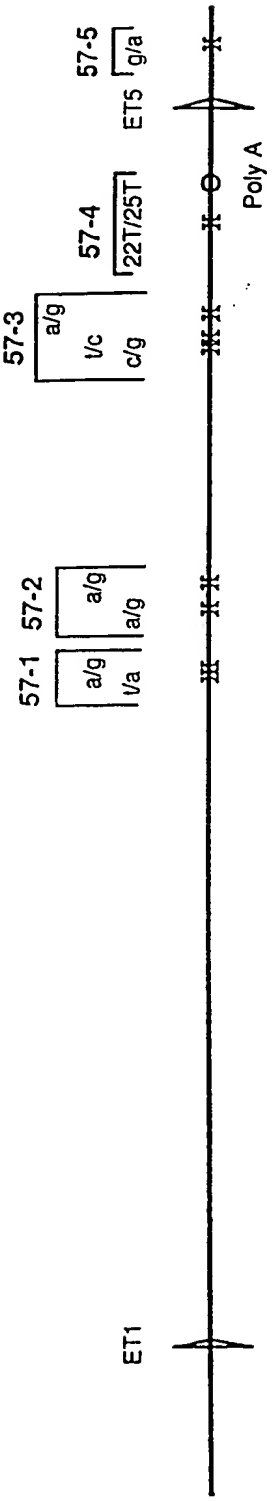


Figure 9

Fig.10.

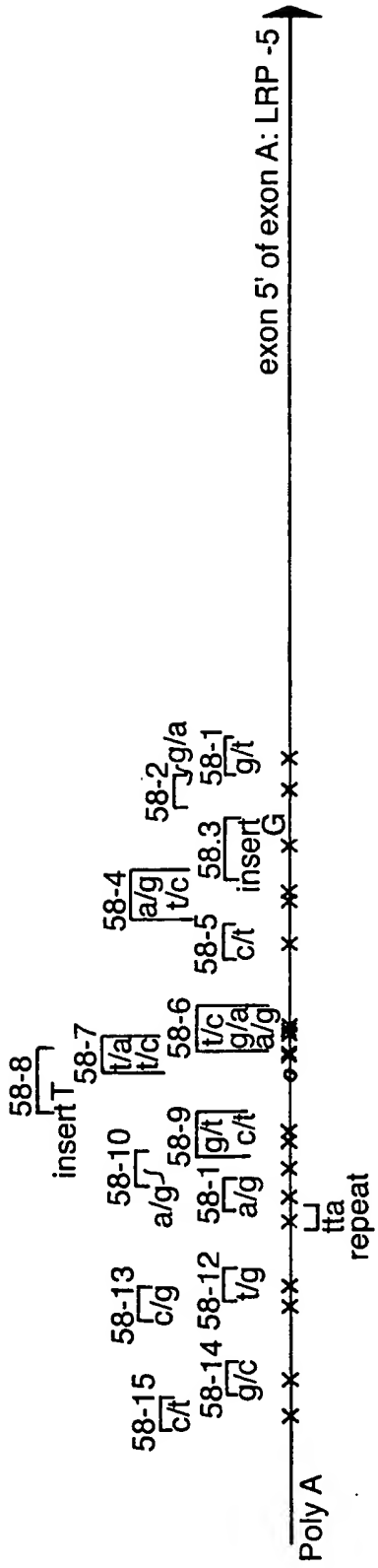

exon 5' of exon A: LRP -5
58-8
insert T
58-7
t/a
t/c
58-6
t/c
g/a
a/g
58-5
c/t
58-4
a/g
t/c
58.3
insert G
58-2
r g/a
58-1
g/t
58-10
a/g
58-9
g/t
c/t
58-1
a/g
58-13
c/g
58-12
t/g
58-14
g/c
58-15
c/t
tta
repeat
Poly A

Figure 11(a)

GAGAGGACACCGCATTCTTCTTCTCCAGAGGATGCAGCAGCAAGGCGCCATC
TTGAAACCAGAGACCAAACCAACCAGCAWTTTTGTCTTGAACTTCCCAGCC
TCCACAACTAATATAAACCCCATGAGGGCAGAGGCGTTCAGCCTGACTCCAG
CCTGGCAAAGCTGTCACAAATCTGGAGGAACACACACGTTCACGGGCACTCA
GTTCTGTGAGCCTCGCCGCTCCTGCTATTTGCCAACCGCCGGGACGTACGGCTG
GTGGACGCCGGCGGAGTCAAGCTGGAGTCCACCATCGTGGTCAGCGGCCTGGA
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TGGCCAACCTCAATGGCACATCCCGGAAGGTGCTCTTCTGGCAGGACCTTGAC
CAGCCGAGGGCCATCGCCTTGGACCCCGCTCACGGGTACATGTACTGGACAGA
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CCCTTCGCCCTGACGCTCTCCGGGGACACTCTGTACTGGACAGACTGGCAGACC
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GGGCCTCATGTACTGGACAGACTGGGGAGAGAACCCTAAAATCGAGTGTGCC
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AGACAGACAAGATCGAGGTGATCAATGTTGATGGGACGAAGAGGCGGACC
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AGGCCAGCCGGGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAA
GCTGTGAATGTGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACG
GGGGGTGCAGCCACCTGTGCTTCTTCACACCCCACGCAACCCGGTGTGGCTGCCC
CATCGGCCTGGAGCTGCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCT

Figure 11(a), Ctd.

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ATCTGCCTGCCCAACCAGTTCCGGTGTGCGAGCGGCCAGTGTGTCCTCATCAA
ACAGCAGTGCGACTCCTTCCCCGACTGTATCGACGGCTCCGACGAGCTCATGT

Figure 11 (a), Ctd.

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GTCACAGGGGCCTCGTCCAGCAGCTCGTCCAGCACGAAGGCCACGCTGTACCCG
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Figure 11(b)

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AGCTTCATCCACCGTGCCAACCTGGACGGCTCGTTCCGGCAGAAGGTGGTGGA
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ACAGACTGGCAGACCCGCTCCATCCATGCCTGCAACAAGCGCACTGGGGGGA
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CCCCACGGGTGTGCAGCTGCAGGACAACGGCAGGACGTGTAAGGCAGGAGCC
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GGCACCTCCCGCAAGATCCTGGTGTCGGAGGACCTGGACGAGCCCCGAGCCATC
GCACTGCACCCCGTGATGGGCCTCATGTACTGGACAGACTGGGGAGAGAACCC
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GGGTGCACAAGGTCAAGGCCAGCCGGGACGTCATCATTGACCAGCTGCCCGAC
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TGGGTCAGGAGCGGGTCGTGATTGCCGACGATCTCCCGCACCCGTTCGGTCTGA

Figure 11(b), ctd,

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CTGTACTTCACCAACATGCAGGACCGGGCAGCCAAGATCGAACGCGCAGCCC
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TGGATCGACCGCCAGCAGCAGATGATCGAGCGTGTGGAGAAGACCACCGGGG
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TCATGCCCAGTCCACCTCGTGCTCCTGCAGAACCTGCTGACCTGTGGAGAGCCG
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GGAGGGCTGCCCCGTGTGCTCCGCCGCCCAGTTCCCCTGCGCGCGGGGTCAGTGT
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GAGGCGGACTGTGACGCCATCTGCCTGCCCAACCAGTTCCGGTGTGCGAGCGGC
CAGTGTGTCCTCATCAAACAGCAGTGCGACTCCTTCCCCGACTGTATCGACGG
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CAGCACCGACGTGTGTGACAGCGACTACAGCGCCAGCCGCTGGAAGGCCAGCA

Figure 11(b), Ctd,

AGTACTACCTGGATTTGAACTCGGACTCAGACCCCTATCCACCCCCACCCACG
CCCCACAGCCAGTACCTGTCGGCGGAGGACAGCTGCCCGCCCTCGCCCGCCACCG
AGAGGAGCTACTTCCATCTCTTCCCGCCCCCTCCGTCCCCCTGCACGGACTCATC
C

Figure 11(c)

MYWTDWGETPRIERAGMDGSTRKIIVDSDIYWPNGLTIDLEEQKLYWADAK
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ARNLYWTDTGTDRIEVTRLNGTSRKILVSEDLDEPRAIALHPVMGLMYWTD
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LSDMKTCIVPEAFLVFTSRAAIHRISLETNNNDVAIPLTGVKEASALDFDVS
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SPPTTFLLFSQKSAISRMIPDDQHSPDLILPLHGLRNVKAIDYDPLDKFIYWV
DGRQNIKRAKDDGTQPFVLTSLSQGQNPDRQPHDLSIDIYSRTLFWTCEATNTI
NVHRLSGEAMGVVLRGDRDKPRAIVVNAERGYLYFTNMQDRAAKIERAAL
DGTEREVLFTTGLIRPVALVVDNTLGKLFWVDADLKRIESCDLSGANRLTLE
DANIVQPLGLTILGKHLYWIDRQQQMIERVEKTTGDKRTRIQGRVAHLTGIH
AVEEVSLEEFSAHPCARDNGGCSHICIAKGDGTPRCSCPVHLVLLQNLLTCGE
PPTCSPDQFACATGEIDCIPGAWRCDGFPECDDQSDEEGCPVCSAAQFPCARGQ
CVDLRLRCDGEADCQDRSDEADCDAICLPNQFRCASGQCVLIKQQCDSFPDCIDG
SDELMCEITKPPSDDSPAHSSAIGPVIGIILSLFVMGGVYFVCQRVVCQRYAG
ANGPFPHEYVSGTPHVPLNFIAPGGSQHGPFTGIACGKSMMSSVSLMGGRGG
VPLYDRNHVTGASSSSSSSTKATLYPPILNPPPSPATDPSLYNMDMFYSSNIP
ATVRPYRPYIIRGMAPPTTPCSTDVCDSDYSASRWKASKYYLDLNSDSDPYP
PPPTPHSQYLSAEDSCPPSPATERSYFHLFPPPPSPCTDSS

Figure 12(a)

TAAATGGCTTGGCAAAGGGAGTTCATTCCTTTTAGCGCTTCCATCTTCTGCA
GTGAGAGGACACCGCATTCTTCTTCTCCAGAGGATGCAGCAGCAAGGCGCCA
TCTTGAAACCAGAGACCAAACCAACCAGCAACTTCGTCTTGAACTTCCCAGC
CTCCACAACTCCTCGCCGCTCCTGCTATTTGCCAACCGCCGGGACGTACGGCTG
GTGGACGCCGGCGGAGTCAAGCTGGAGTCCACCATCGTGGTCAGCGGCCTGGA
GGATGCGGCCGCAGTGGACTTCCAGTTTTCCAAGGGAGCCGTGTACTGGACAG
ACGTGAGCGAGGAGGCCATCAAGCAGACCTACCTGAACCAGACGGGGGCCGC
CGTGCAGAACGTGGTCATCTCCGGCCTGGTCTCTCCCGACGGCCTCGCCTGCGAC
TGGGTGGGCAAGAAGCTGTACTGGACGGACTCAGAGACCAACCGCATCGAGG
TGGCCAACCTCAATGGCACATCCCGGAAGGTGCTCTTCTGGCAGGACCTTGAC
CAGCCGAGGGCCATCGCCTTGGACCCCGCTCACGGGTACATGTACTGGACAGA
CTGGGGTGAGACGCCCCGGATTGAGCGGGCAGGGATGGATGGCAGCACCCGGA
AGATCATTGTGGACTCGGACATTTACTGGCCCAATGGACTGACCATCGACCT
GGAGGAGCAGAAGCTCTACTGGGCTGACGCCAAGCTCAGCTTCATCCACCGTG
CCAACCTGGACGGCTCGTTCCGGCAGAAGGTGGTGGAGGGCAGCCTGACGCAC
CCCTTCGCCCTGACGCTCTCCGGGGACACTCTGTACTGGACAGACTGGCAGACC
CGCTCCATCCATGCCTGCAACAAGCGCACTGGGGGGAAGAGGAAGGAGATCC
TGAGTGCCCTCTACTCACCCATGGACATCCAGGTGCTGAGCCAGGAGCGGCAG
CCTTTCTTCCACACTCGCTGTGAGGAGGACAATGGCGGCTGCTCCCACCTGTGC
CTGCTGTCCCCAAGCGAGCCTTTCTACACATGCGCCTGCCCCACGGGTGTGCAG
CTGCAGGACAACGGCAGGACGTGTAAGGCAGGAGCCGAGGAGGTGCTGCTGC
TGGCCCGGCGGACGGACCTACGGAGGATCTCGCTGGACACGCCGGACTTTACCG
ACATCGTGCTGCAGGTGGACGACATCCGGCACGCCATTGCCATCGACTACGAC
CCGCTAGAGGGCTATGTCTACTGGACAGATGACGAGGTGCGGGCCATCCGCAG
GGCGTACCTGGACGGGTCTGGGGCGCAGACGCTGGTCAACACCGAGATCAACG
ACCCCGATGGCATCGCGGTCGACTGGGTGGCCCGAAACCTCTACTGGACCGAC
ACGGGCACGGACCGCATCGAGGTGACGCGCCTCAACGGCACCTCCCGCAAGAT
CCTGGTGTCGGAGGACCTGGACGAGCCCCGAGCCATCGCACTGCACCCCGTGAT
GGGCCTCATGTACTGGACAGACTGGGGAGAGAACCCTAAAATCGAGTGTGCC
AACTTGGATGGGCAGGAGCGGCGTGTGCTGGTCAATGCCTCCCTCGGGTGGCCC
AACGGCCTGGCCCTGGACCTGCAGGAGGGGAAGCTCTACTGGGGAGACGCCA
AGACAGACAAGATCGAGGTGATCAATGTTGATGGGACGAAGAGGCGGACC
CTCCTGGAGGACAAGCTCCCGCACATTTTCGGGTTCACGCTGCTGGGGGACTTC
ATCTACTGGACTGACTGGCAGCGCCGCAGCATCGAGCGGGTGCACAAGGTCA
AGGCCAGCCGGGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAA
GCTGTGAATGTGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACG
GGGGGTGCAGCCACCTGTGCTTCTTCACACCCCACGCAACCCGGTGTGGCTGCCC
CATCGGCCTGGAGCTGCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCT
TCTTGGTCTTCACCAGCAGAGCCGCCATCCACAGGATCTCCCTCGAGACCAAT

Figure 12(a), Ctd.

AACAACGACGTGGCCATCCCGCTCACGGGCGTCAAGGAGGCCTCAGCCCTGGA
CTTTGATGTGTCCAACAACCACATCTACTGGACAGACGTCAGCCTGAAGACC
ATCAGCCCGCGCCTTCATGAACGGGAGCTCGGTGGAGCACGTGGTGGAGTTTGG
CCTTGACTACCCCGAGGGCATGGCCGTTGACTGGATGGGCAAGAACCTCTACT
GGGCCGACACTGGGACCAACAGAATCGAAGTGGCGCGGCTGGACGGGCAGTT
CCGGCAAGTCCTCGTGTGGAGGGACTTGGACAACCCGAGGTCGCTGGCCCTGG
ATCCCACCAAGGGCTACATCTACTGGACCGAGTGGGGCGGCAAGCCGAGGAT
CGTGCGGGCCTTCATGGACGGGACCAACTGCATGACGCTGGTGGACAAGGTGG
GCCGGGCCAACGACCTCACCATTGACTACGCTGACCAGCGCCTCTACTGGACCG
ACCTGGACACCAACATGATCGAGTCGTCCAACATGCTGGGTCAGGAGCGGGT
CGTGATTGCCGACGATCTCCCGCACCCGTTCGGTCTGACGCAGTACAGCGATT
ATATCTACTGGACAGACTGGAATCTGCACAGCATTGAGCGGGCCGACAAGA
CTAGCGGCCGGAACCGCACCCTCATCCAGGGCCACCTGGACTTCGTGATGGAC
ATCCTGGTGTTCCACTCCTCCCGCCAGGATGGCCTCAATGACTGTATGCACAA
CAACGGGCAGTGTGGGCAGCTGTGCCTTGCCATCCCCGGCGGCCACCGCTGCGGC
TGCGCCTCACACTACACCCTGGACCCCAGCAGCCGCAACTGCAGCCCGCCCACC
ACCTTCTTGCTGTTCAGCCAGAAATCTGCCATCAGTCGGATGATCCCGGACGA
CCAGCACAGCCCGGATCTCATCCTGCCCCTGCATGGACTGAGGAACGTCAAAG
CCATCGACTATGACCCACTGGACAAGTTCATCTACTGGGTGGATGGGCGCCA
GAACATCAAGCGAGCCAAGGACGACGGGACCCAGCCCTTTGTTTTGACCTCT
CTGAGCCAAGGCCAAAACCCAGACAGGCAGCCCCACGACCTCAGCATCGACA
TCTACAGCCGGACACTGTTCTGGACGTGCGAGGCCACCAATACCATCAACGTC
CACAGGCTGAGCGGGGAAGCCATGGGGGTGGTGCTGCGTGGGGACCGCGACAA
GCCCAGGGCCATCGTCGTCAACGCGGAGCGAGGGTACCTGTACTTCACCAACA
TGCAGGACCGGGCAGCCAAGATCGAACGCGCAGCCCTGGACGGCACCGAGCGC
GAGGTCCTCTTCACCACCGGCCTCATCCGCCCTGTGGCCCTGGTGGTAGACAAC
ACACTGGGCAAGCTGTTCTGGGTGGACGCGGACCTGAAGCGCATTGAGAGCT
GTGACCTGTCAGGGGCCAACCGCCTGACCCTGGAGGACGCCAACATCGTGCAG
CCTCTGGGCCTGACCATCCTTGGCAAGCATCTCTACTGGATCGACCGCCAGCAG
CAGATGATCGAGCGTGTGGAGAAGACCACCGGGGACAAGCGGACTCGCATCC
AGGGCCGTGTCGCCCACCTCACTGGCATCCATGCAGTGGAGGAAGTCAGCCTG
GAGGAGTTCTCAGCCCACCCATGTGCCCGTGACAATGGTGGCTGCTCCCACAT
CTGTATTGCCAAGGGTGATGGGACACCACGGTGCTCATGCCCAGTCCACCTCG
TGCTCCTGCAGAACCTGCTGACCTGTGGAGAGCCGCCCACCTGCTCCCCGGACC
AGTTTGCATGTGCCACAGGGGAGATCGACTGTATCCCCGGGGCCTGGCGCTGT
GACGGCTTTCCCGAGTGCGATGACCAGAGCGACGAGGAGGGCTGCCCCGTGTG
CTCCGCCGCCCAGTTCCCCTGCGCCGCGGGGTCAGTGTGTGGACCTGCGCCTGCGCT
GCGACGGCGAGGCAGACTGTCAGGACCGCTCAGACGAGGCGGACTGTGACGCC
ATCTGCCTGCCCAACCAGTTCCGGTGTGCGAGCGGCCAGTGTGTCCTCATCAA
ACAGCAGTGCGACTCCTTCCCCGACTGTATCGACGGCTCCGACGAGCTCATGT
GTGAAATCACCAAGCCGCCCTCAGACGACAGCCCGGCCCACAGCAGTGCCATC

Figure 12(a), Ctd.

GGGCCCGTCATTGGCATCATCCTCTCTCTCTTCGTCATGGGTGGTGTCTATTTT
GTGTGCCAGCGCGTGGTGTGCCAGCGCTATGCGGGGGCCAACGGGCCCTTCCCGC
ACGAGTATGTCAGCGGGACCCCGCACGTGCCCCTCAATTTCATAGCCCCGGGCG
GTTCCCAGCATGGCCCCTTCACAGGCATCGCATGCGGAAAGTCCATGATGAGC
TCCGTGAGCCTGATGGGGGGCCGGGGCGGGGTGCCCCTCTACGACCGGAACCAC
GTCACAGGGGCCTCGTCCAGCAGCTCGTCCAGCACGAAGGCCACGCTGTACCCG
CCGATCCTGAACCCGCCGCCCTCCCCGGCCACGGACCCCTCCCTGTACAACATGG
ACATGTTCTACTCTTCAAACATTCCGGCCACTGCGAGACCGTACAGGCCCTAC
ATCATTCGAGGAATGGCGCCCCCGACGACGCCCTGCAGCACCGACGTGTGTGA
CAGCGACTACAGCGCCAGCCGCTGGAAGGCCAGCAAGTACTACCTGGATTTG
AACTCGGACTCAGACCCCTATCCACCCCCACCCACGCCCCACAGCCAGTACCTG
TCGGCGGAGGACAGCTGCCCGCCCTCGCCCGCCACCGAGAGGAGCTACTTCCAT
CTCTTCCCGCCCCCTCCGTCCCCCTGCACGGACTCATCCTGACCTCGGCCGGGCCA
CTCTGGCTTCTCTGTGCCCCTGTAAATAGTTTTAAATATGAACAAAGAAAA
AAATATATTTTATGATTTAAAAAATAAATATAATTGGGATTTTAAAA
ACATGAGAAATGTGAACTGTGATGGGGTGGGCAGGGCTGGGAGAACTTTGT
ACAGTGGAACAAATATTTATAAACTTAATTTTGTAAAACAG

Figure 12(b)

TAAAATGGCTTGGCAAAGGGAGTTCATTCCTTTTAGCGCTTCCATCTTCTGC
AGTGAGAGGACACCGCATTCTTCTTCTCCAGAGGATGCAGCAGCAAGGCGCC
ATCTTGAAACCAGAGACCAAACCAACCAGCAACTTCGTCTTGAACTTCCCA
GCCTCCACAACT

Figure 12(c)

ATGGCTTGGCAAAGGGAGTTCATTCCTTTTAGCGCTTCCATCTTCTGCAGTGA
GAGGACACCGCATTCTTCTTCTCCAGAGGATGCAGCAGCAAGGCGCCATCTTG
AAACCAGAGACCAAACCAACCAGCAACTTCGTCTTGAACTTCCCAGCCTCCA
CAACTCCTCGCCGCTCCTGCTATTTGCCAACCGCCGGGACGTACGGCTGGTGGA
CGCCGGCGGAGTCAAGCTGGAGTCCACCATCGTGGTCAGCGGCCTGGAGGATG
CGGCCGCAGTGGACTTCCAGTTTTCCAAGGGAGCCGTGTACTGGACAGACGTG
AGCGAGGAGGCCATCAAGCAGACCTACCTGAACCAGACGGGGGCCGCCGTGC
AGAACGTGGTCATCTCCGGCCTGGTCTCTCCCGACGGCCTCGCCTGCGACTGGGT
GGGCAAGAAGCTGTACTGGACGGACTCAGAGACCAACCGCATCGAGGTGGCC
AACCTCAATGGCACATCCCGGAAGGTGCTCTTCTGGCAGGACCTTGACCAGCC
GAGGGCCATCGCCTTGGACCCCGCTCACGGGTACATGTACTGGACAGACTGGG
GTGAGACGCCCCGGATTGAGCGGGCAGGGATGGATGGCAGCACCCGGAAGAT
CATTGTGGACTCGGACATTTACTGGCCCAATGGACTGACCATCGACCTGGAG
GAGCAGAAGCTCTACTGGGCTGACGCCAAGCTCAGCTTCATCCACCGTGCCAA
CCTGGACGGCTCGTTCCGGCAGAAGGTGGTGGAGGGCAGCCTGACGCACCCCTT
CGCCCTGACGCTCTCCGGGGACACTCTGTACTGGACAGACTGGCAGACCCGCTC
CATCCATGCCTGCAACAAGCGCACTGGGGGGAAGAGGAAGGAGATCCTGAG
TGCCCTCTACTCACCCATGGACATCCAGGTGCTGAGCCAGGAGCGGCAGCCTT
TCTTCCACACTCGCTGTGAGGAGGACAATGGCGGCTGCTCCCACCTGTGCCTGC
TGTCCCCAAGCGAGCCTTTCTACACATGCGCCTGCCCCACGGGTGTGCAGCTGC
AGGACAACGGCAGGACGTGTAAGGCAGGAGCCGAGGAGGTGCTGCTGCTGGC
CCGGCGGACGGACCTACGGAGGATCTCGCTGGACACGCCGGACTTTACCGACA
TCGTGCTGCAGGTGGACGACATCCGGCACGCCATTGCCATCGACTACGACCCGC
TAGAGGGCTATGTCTACTGGACAGATGACGAGGTGCGGGCCATCCGCAGGGC
GTACCTGGACGGGTCTGGGGCGCAGACGCTGGTCAACACCGAGATCAACGACC
CCGATGGCATCGCGGTCGACTGGGTGGCCCGAAACCTCTACTGGACCGACACG
GGCACGGACCGCATCGAGGTGACGCGCCTCAACGGCACCTCCCGCAAGATCCT
GGTGTCGGAGGACCTGGACGAGCCCCGAGCCATCGCACTGCACCCCGTGATGGG
CCTCATGTACTGGACAGACTGGGGAGAGAACCCTAAAATCGAGTGTGCCAA
CTTGGATGGGCAGGAGCGGCGTGTGCTGGTCAATGCCTCCCTCGGGTGGCCCAA
CGGCCTGGCCCTGGACCTGCAGGAGGGGAAGCTCTACTGGGGAGACGCCAAGA
CAGACAAGATCGAGGTGATCAATGTTGATGGGACGAAGAGGCGGACCCTCC
TGGAGGACAAGCTCCCGCACATTTTCGGGTTCACGCTGCTGGGGGACTTCATC
TACTGGACTGACTGGCAGCGCCGCAGCATCGAGCGGGTGCACAAGGTCAAGG
CCAGCCGGGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAAGCT
GTGAATGTGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACGGGG
GGTGCAGCCACCTGTGCTTCTTCACACCCCACGCAACCCCGGTGTGGCTGCCCCA
TCGGCCTGGAGCTGCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCTTC
TTGGTCTTCACCAGCAGAGCCGCCATCCACAGGATCTCCCTCGAGACCAATA

Figure 12(c), Ctd.

ACAACGACGTGGCCATCCCGCTCACGGGCGTCAAGGAGGCCTCAGCCCTGGAC
TTTGATGTGTCCAACAACCACATCTACTGGACAGACGTCAGCCTGAAGACC
ATCAGCCGCGCCTTCATGAACGGGAGCTCGGTGGAGCACGTGGTGGAGTTTGG
CCTTGACTACCCCGAGGGCATGGCCGTTGACTGGATGGGCAAGAACCTCTACT
GGGCCGACACTGGGACCAACAGAATCGAAGTGGCGCGGCTGGACGGGCAGTT
CCGGCAAGTCCTCGTGTGGAGGGACTTGGACAACCCGAGGTCGCTGGCCCTGG
ATCCCACCAAGGGCTACATCTACTGGACCGAGTGGGGCGGCAAGCCGAGGAT
CGTGCGGGCCTTCATGGACGGGACCAACTGCATGACGCTGGTGGACAAGGTGG
GCCGGGCCAACGACCTCACCATTGACTACGCTGACCAGCGCCTCTACTGGACCG
ACCTGGACACCAACATGATCGAGTCGTCCAACATGCTGGGTCAGGAGCGGGT
CGTGATTGCCGACGATCTCCCGCACCCGTTCGGTCTGACGCAGTACAGCGATT
ATATCTACTGGACAGACTGGAATCTGCACAGCATTGAGCGGGCCGACAAGA
CTAGCGGCCGGAACCGCACCCTCATCCAGGGCCACCTGGACTTCGTGATGGAC
ATCCTGGTGTTCCACTCCTCCCGCCAGGATGGCCTCAATGACTGTATGCACAA
CAACGGGCAGTGTGGGCAGCTGTGCCTTGCCATCCCCGGCGGCCACCGCTGCGGC
TGCGCCTCACACTACACCCTGGACCCCAGCAGCCGCAACTGCAGCCCGCCCACC
ACCTTCTTGCTGTTCAGCCAGAAATCTGCCATCAGTCGGATGATCCCGGACGA
CCAGCACAGCCCGGATCTCATCCTGCCCCTGCATGGACTGAGGAACGTCAAAG
CCATCGACTATGACCCACTGGACAAGTTCATCTACTGGGTGGATGGGCGCCA
GAACATCAAGCGAGCCAAGGACGACGGGACCCAGCCCTTTGTTTTGACCTCT
CTGAGCCAAGGCCAAAACCCAGACAGGCAGCCCCACGACCTCAGCATCGACA
TCTACAGCCGGACACTGTTCTGGACGTGCGAGGCCACCAATACCATCAACGTC
CACAGGCTGAGCGGGGAAGCCATGGGGGTGGTGCTGCGTGGGGACCGCGACAA
GCCCAGGGCCATCGTCGTCAACGCGGAGCGAGGGTACCTGTACTTCACCAACA
TGCAGGACCGGGCAGCCAAGATCGAACGCGCAGCCCTGGACGGCACCGAGCGC
GAGGTCCTCTTCACCACCGGCCTCATCCGCCCTGTGGCCCTGGTGGTAGACAAC
ACACTGGGCAAGCTGTTCTGGGTGGACGCGGACCTGAAGCGCATTGAGAGCT
GTGACCTGTCAGGGGCCAACCGCCTGACCCTGGAGGACGCCAACATCGTGCAG
CCTCTGGGCCTGACCATCCTTGGCAAGCATCTCTACTGGATCGACCGCCAGCAG
CAGATGATCGAGCGTGTGGAGAAGACCACCGGGGACAAGCGGACTCGCATCC
AGGGCCGTGTCGCCCACCTCACTGGCATCCATGCAGTGGAGGAAGTCAGCCTG
GAGGAGTTCTCAGCCCACCCATGTGCCCGTGACAATGGTGGCTGCTCCCACAT
CTGTATTGCCAAGGGTGATGGGACACCACGGTGCTCATGCCCAGTCCACCTCG
TGCTCCTGCAGAACCTGCTGACCTGTGGAGAGCCGCCCACCTGCTCCCCGGACC
AGTTTGCATGTGCCACAGGGGAGATCGACTGTATCCCCGGGGCCTGGCGCTGT
GACGGCTTTCCCGAGTGCGATGACCAGAGCGACGAGGAGGGCTGCCCCGTGTG
CTCCGCCGCCCAGTTCCCCTGCGCGCGGGGTCAGTGTGTGGACCTGCGCCTGCGCT
GCGACGGCGAGGCAGACTGTCAGGACCGCTCAGACGAGGCGGACTGTGACGCC
ATCTGCCTGCCCAACCAGTTCCGGTGTGCGAGCGGCCAGTGTGTCCTCATCAA
ACAGCAGTGCGACTCCTTCCCCGACTGTATCGACGGCTCCGACGAGCTCATGT
GTGAAATCACCAAGCCGCCCTCAGACGACAGCCCGGCCCACAGCAGTGCCATC

Figure 12(C), Ctd.

GGGCCCGTCATTGGCATCATCCTCTCTCTCTTCGTCATGGGTGGTGTCTATTTT
GTGTGCCAGCGCGTGGTGTGCCAGCGCTATGCGGGGGCCAACGGGCCCTTCCCGC
ACGAGTATGTCAGCGGGACCCCGCACGTGCCCCTCAATTTCATAGCCCCGGGCG
GTTCCCAGCATGGCCCCTTCACAGGCATCGCATGCGGAAAGTCCATGATGAGC
TCCGTGAGCCTGATGGGGGGCCGGGGCGGGGTGCCCCTCTACGACCGGAACCAC
GTCACAGGGGCCTCGTCCAGCAGCTCGTCCAGCACGAAGGCCACGCTGTACCCG
CCGATCCTGAACCCGCCGCCCTCCCCGGCCACGGACCCCTCCCTGTACAACATGG
ACATGTTCTACTCTTCAAACATTCCGGCCACTGCGAGACCGTACAGGCCCTAC
ATCATTCGAGGAATGGCGCCCCCGACGACGCCCTGCAGCACCGACGTGTGTGA
CAGCGACTACAGCGCCAGCCGCTGGAAGGCCAGCAAGTACTACCTGGATTTG
AACTCGGACTCAGACCCCTATCCACCCCCACCCACGCCCCACAGCCAGTACCTG
TCGGCGGAGGACAGCTGCCCGCCCTCGCCCGCCACCGAGAGGAGCTACTTCCAT
CTCTTCCCGCCCCCTCCGTCCCCCTGCACGGACTCATCC

Figure 12(d)

MAWQREFIPFSASIFCSERTPHSSSPEDAAARRHLETRDQTNQQLRLELPSLH
NSSPLLLFANRRDVRLVDAGGVKLESTIVVSGLEDAAAVDFQFSKGAVYWT
DVSEEAIKQTYLNQTGAAVQNVVISGLVSPDGLACDWVGKKLYWTDSETNR
IEVANLNGTSRKVLFWQDLDQPRAIALDPAHGYMYWTDWGETPRIERAGM
DGSTRKIIVDSDIYWPNGLTIDLEEQKLYWADAKLSFIHRANLDGSFRQKVVE
GSLTHPFALTLSGDTLYWTDWQTRSIHACNKRTGGKRKEILSALYSPMDIQV
LSQERQPFFHTRCEEDNGGCSHLCLLSPSEPFYTCACPTGVQLQDNGRTCKAGA
EEVLLLARRTDLRRISLDTPDFTDIVLQVDDIRHAIAIDYDPLEGYVYWTDDE
VRAIRRAYLDGSGAQTLVNTEINDPDGIAVDWVARNLYWTDTGTDRIEVTR
LNGTSRKILVSEDLDEPRAIALHPVMGLMYWTDWGENPKIECANLDGQERR
VLVNASLGWPNGLALDLQEGKLYWGDAKTDKIEVINVDGTKRRTLLEDKLP
HIFGFTLLGDFIYWTDWQRRSIERVHKVKASRDVIIDQLPDLMGLKAVNVA
KVVGTNPCADRNGGCSHLCFFTPHATRCGCPIGLELLSDMKTCIVPEAFLVFT
SRAAIHRISLETNNNDVAIPLTGVKEASALDFDVSNNHIYWTDVSLKTISRA
FMNGSSVEHVVEFGLDYPEGMAVDWMGKNLYWADTGTNRIEVARLDGQFR
QVLVWRDLDNPRSLALDPTKGYIYWTEWGGKPRIVRAFMDGTNCMTLVDK
VGRANDLTIDYADQRLYWTDLDTNMIESSNMLGQERVVIADDLPHPFGLTQ
YSDYIYWTDWNLHSIERADKTSGRNRTLIQGHLDFVMDILVFHSSRQDGLND
CMHNNGQCGQLCLAIPGGHRCGCASHYTLDPSSRNCSPPTTFLLFSQKSAISR
MIPDDQHSPDLILPLHGLRNVKAIDYDPLDKFIYWVDGRQNIKRAKDDGTQPF
VLTSLSQGQNPDRQPHDLSIDIYSRTLFWTCEATNTINVHRLSGEAMGVVLR
GDRDKPRAIVVNAERGYLYFTNMQDRAAKIERAALDGTEREVLFTTGLIRPV
ALVVDNTLGKLFWVDADLKRIESCDLSGANRLTLEDANIVQPLGLTILGKHL
YWIDRQQQMIERVEKTTGDKRTRIQGRVAHLTGIHAVEEVSLEEFSAHPCAR
DNGGCSHICIAKGDGTPRCSCPVHLVLLQNLLTCGEPPTCSPDQFACATGEIDCI
PGAWRCDGFPECDDQSDEEGCPVCSAAQFPCARGQCVDLRLRCDGEADCQDRS
DEADCDAICLPNQFRCASGQCVLIKQQCDSFPDCIDGSDELMCEITKPPSDDSPA
HSSAIGPVIGIILSLFVMGGVYFVCQRVVCQRYAGANGPFPHEYVSGTPHVP
LNFIAPGGSQHGPFTGIACGKSMMSSVSLMGGRGGVPLYDRNHVTGASSSSS
SSTKATLYPPILNPPPSPATDPSLYNMDMFYSSNIPATARPYRPYIIRGMAP
PTTPCSTDVCDSDYSASRWKASKYYLDLNSDSDPYPPPPTPHSQYLSAEDSCP
PSPATERSYFHLFPPPPSPCTDSS

Figure 12(e)

TATAAAATGGCTTGGCAAAGGGAGTTCATTCCTTTTAGCGCTTCCATCTTCT
GCAGTGAGAGGACACCGCATTCTTCTTCTCCAGAGGATG

Figure 13

TAAGAGTATAAAGGGCTCCTGAGACCAAAAAGGTTGAGAACCAGTGCTTT
AAAGCTTGATGTTTCTCAGGGTTTCATCCTTTGTGGATTAATGCCCATTATA
AAATGGCTTGGCAAAGGGAGTTCATTCCTTTTAGCGCTTCCATCTTCTGCAG
TGAGAGGACACCGCATTCTTCTTCTCCAGAGGATGCAGCAGCAAGGCGCCAT
CTTGAAACCAGAGACCAAACCAACCAGCAACTTCGTCTTGAACTTCCCAGCC
TCCACAACTCAGCAGTCTGTGCAGGACCCTGTGAGCAGAGCCGCAGCCTCGCC
GCTCCTGCTATTTGCCAACCGCCGGGACGTACGGCTGGTGGACGCCGGCGGAGT
CAAGCTGGAGTCCACCATCGTGGTCAGCGGCCTGGAGGATGCGGCCGCAGTGG
ACTTCCAGTTTTCCAAGGGAGCCGTGTACTGGACAGACGTGAGCGAGGAGGC
CATCAAGCAGACCTACCTGAACCAGACGGGGGCCGCCGTGCAGAACGTGGTC
ATCTCCGGCCTGGTCTCTCCCGACGGCCTCGCCTGCGACTGGGTGGGCAAGAAG
CTGTACTGGACGGACTCAGAGACCAACCGCATCGAGGTGGCCAACCTCAATG
GCACATCCCGGAAGGTGCTCTTCTGGCAGGACCTTGACCAGCCGAGGGCCATC
GCCTTGGACCCCGCTCACGGGTACATGTACTGGACAGACTGGGGTGAGACGCC
CCGGATTGAGCGGGCAGGGATGGATGGCAGCACCCGGAAGATCATTGTGGAC
TCGGACATTTACTGGCCCAATGGACTGACCATCGACCTGGAGGAGCAGAAGC
TCTACTGGGCTGACGCCAAGCTCAGCTTCATCCACCGTGCCAACCTGGACGGCT
CGTTCCGGCAGAAGGTGGTGGAGGGCAGCCTGACGCACCCCTTCGCCCTGACGC
TCTCCGGGGACACTCTGTACTGGACAGACTGGCAGACCCGCTCCATCCATGCCT
GCAACAAGCGCACTGGGGGGAAGAGGAAGGAGATCCTGAGTGCCCTCTACTC
ACCCATGGACATCCAGGTGCTGAGCCAGGAGCGGCAGCCTTTCTTCCACACTC
GCTGTGAGGAGGACAATGGCGGCTGCTCCCACCTGTGCCTGCTGTCCCCAAGCG
AGCCTTTCTACACATGCGCCTGCCCCACGGGTGTGCAGCTGCAGGACAACGGC
AGGACGTGTAAGGCAGGAGCCGAGGAGGTGCTGCTGCTGGCCCGGCGGACGGA
CCTACGGAGGATCTCGCTGGACACGCCGGACTTTACCGACATCGTGCTGCAGG
TGGACGACATCCGGCACGCCATTGCCATCGACTACGACCCGCTAGAGGGCTAT
GTCTACTGGACAGATGACGAGGTGCGGGCCATCCGCAGGGCGTACCTGGACGG
GTCTGGGGCGCAGACGCTGGTCAACACCGAGATCAACGACCCCGATGGCATCG
CGGTCGACTGGGTGGCCCGAAACCTCTACTGGACCGACACGGGCACGGACCGC
ATCGAGGTGACGCGCCTCAACGGCACCTCCCGCAAGATCCTGGTGTCGGAGGA
CCTGGACGAGCCCCGAGCCATCGCACTGCACCCCGTGATGGGCCTCATGTACTG
GACAGACTGGGGAGAGAACCCTAAAATCGAGTGTGCCAACTTGGATGGGCA
GGAGCGGCGTGTGCTGGTCAATGCCTCCCTCGGGTGGCCCAACGGCCTGGCCCTG
GACCTGCAGGAGGGGAAGCTCTACTGGGGAGACGCCAAGACAGACAAGATC
GAGGTGATCAATGTTGATGGGACGAAGAGGCGGACCCTCCTGGAGGACAAG
CTCCCGCACATTTTCGGGTTCACGCTGCTGGGGGACTTCATCTACTGGACTGAC
TGGCAGCGCCGCAGCATCGAGCGGGTGCACAAGGTCAAGGCCAGCCGGGACGT
CATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAAGCTGTGAATGTGGCC
AAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACGGGGGGTGCAGCCACC

Figure 13, ctd

TGTGCTTCTTCACACCCCACGCAACCCGGTGTGGCTGCCCCATCGGCCTGGAGCT
GCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCTTCTTGGTCTTCACCA
GCAGAGCCGCCATCCACAGGATCTCCCTCGAGACCAATAACAACGACGTGGC
CATCCCGCTCACGGGCGTCAAGGAGGCCTCAGCCCTGGACTTTGATGTGTCCA
ACAACCACATCTACTGGACAGACGTCAGCCTGAAGACCATCAGCCGCGCCTT
CATGAACGGGAGCTCGGTGGAGCACGTGGTGGAGTTTGGCCTTGACTACCCCG
AGGGCATGGCCGTTGACTGGATGGGCAAGAACCTCTACTGGGCCGACACTGG
GACCAACAGAATCGAAGTGGCGCGGCTGGACGGGCAGTTCCGGCAAGTCCTC
GTGTGGAGGGACTTGGACAACCCGAGGTCGCTGGCCCTGGATCCCACCAAGGG
CTACATCTACTGGACCGAGTGGGGCGGCAAGCCGAGGATCGTGCGGGCCTTCA
TGGACGGGACCAACTGCATGACGCTGGTGGACAAGGTGGGCCGGGCCAACGA
CCTCACCATTGACTACGCTGACCAGCGCCTCTACTGGACCGACCTGGACACCA
ACATGATCGAGTCGTCCAACATGCTGGGTCAGGAGCGGGTCGTGATTGCCGA
CGATCTCCCGCACCCGTTCGGTCTGACGCAGTACAGCGATTATATCTACTGGA
CAGACTGGAATCTGCACAGCATTGAGCGGGCCGACAAGACTAGCGGCCGGAA
CCGCACCCTCATCCAGGGCCACCTGGACTTCGTGATGGACATCCTGGTGTTCCA
CTCCTCCCGCCAGGATGGCCTCAATGACTGTATGCACAACAACGGGCAGTGTG
GGCAGCTGTGCCTTGCCATCCCCGGCGGCCACCGCTGCGGCTGCGCCTCACACTA
CACCCTGGACCCCAGCAGCCGCAACTGCAGCCCGCCCACCACCTTCTTGCTGTTC
AGCCAGAAATCTGCCATCAGTCGGATGATCCCGGACGACCAGCACAGCCCGG
ATCTCATCCTGCCCCTGCATGGACTGAGGAACGTCAAAGCCATCGACTATGA
CCCACTGGACAAGTTCATCTACTGGGTGGATGGGCGCCAGAACATCAAGCGA
GCCAAGGACGACGGGACCCAGCCCTTTGTTTTGACCTCTCTGAGCCAAGGCCA
AAACCCAGACAGGCAGCCCCACGACCTCAGCATCGACATCTACAGCCGGACA
CTGTTCTGGACGTGCGAGGCCACCAATACCATCAACGTCCACAGGCTGAGCGG
GGAAGCCATGGGGGTGGTGCTGCGTGGGGACCGCGACAAGCCCAGGGCCATCG
TCGTCAACGCGGAGCGAGGGTACCTGTACTTCACCAACATGCAGGACCGGGC
AGCCAAGATCGAACGCGCAGCCCTGGACGGCACCGAGCGCGAGGTCCTCTTCA
CCACCGGCCTCATCCGCCCTGTGGCCCTGGTGGTAGACAACACACTGGGCAAGC
TGTTCTGGGTGGACGCGGACCTGAAGCGCATTGAGAGCTGTGACCTGTCAGGG
GCCAACCGCCTGACCCTGGAGGACGCCAACATCGTGCAGCCTCTGGGCCTGACC
ATCCTTGGCAAGCATCTCTACTGGATCGACCGCCAGCAGCAGATGATCGAGC
GTGTGGAGAAGACCACCGGGGACAAGCGGACTCGCATCCAGGGCCGTGTCGCC
CACCTCACTGGCATCCATGCAGTGGAGGAAGTCAGCCTGGAGGAGTTCTCAG
CCCACCCATGTGCCCGTGACAATGGTGGCTGCTCCCACATCTGTATTGCCAAG
GGTGATGGGACACCACGGTGCTCATGCCCAGTCCACCTCGTGCTCCTGCAGAA
CCTGCTGACCTGTGGAGAGCCGCCCACCTGCTCCCCGGACCAGTTTGCATGTGCC
ACAGGGGAGATCGACTGTATCCCCGGGGCCTGGCGCTGTGACGGCTTTCCCGAG
TGCGATGACCAGAGCGACGAGGAGGGCTGCCCCGTGTGCTCCGCCGCCCAGTTC
CCCTGCGCGCGGGGTCAGTGTGTGGACCTGCGCCTGCGCTGCGACGGCGAGGCAG
ACTGTCAGGACCGCTCAGACGAGGCGGACTGTGACGCCATCTGCCTGCCCAAC

Figure 13, ctd.

CAGTTCCGGTGTGCGAGCGGCCAGTGTGTCCTCATCAAACAGCAGTGCGACTC
CTTCCCCCGACTGTATCGACGGCTCCGACGAGCTCATGTGTGAAATCACCAAGC
CGCCCTCAGACGACAGCCCGGCCCACAGCAGTGCCATCGGGCCCGTCATTGGCA
TCATCCTCTCTCTCTTCGTCATGGGTGGTGTCTATTTTGTGTGCCAGCGCGTGG
TGTGCCAGCGCTATGCGGGGGCCAACGGGCCCTTCCCGCACGAGTATGTCAGCG
GGACCCCCGCACGTGCCCCTCAATTTCATAGCCCCGGGCGGTTCCCAGCATGGCCC
CTTCACAGGCATCGCATGCGGAAAGTCCATGATGAGCTCCGTGAGCCTGATG
GGGGGCCGGGGCGGGGTGCCCCTCTACGACCGGAACCACGTCACAGGGGCCTCG
TCCAGCAGCTCGTCCAGCACGAAGGCCACGCTGTACCCGCGGATCCTGAACCCG
CCGCCCTCCCCCGGCCACGGACCCCTCCCTGTACAACATGGACATGTTCTACTCT
TCAAACATTCCGGCCACTGCGAGACCGTACAGGCCCTACATCATTCGAGGAA
TGGCGCCCCCCGACGACGCCCTGCAGCACCGACGTGTGTGACAGCGACTACAGCG
CCAGCCGCTGGAAGGCCAGCAAGTACTACCTGGATTTGAACTCGGACTCAGA
CCCCTATCCACCCCCACCCACGCCCCACAGCCAGTACCTGTCGGCGGAGGACAG
CTGCCCGCCCTCGCCCGCCACCGAGAGGAGCTACTTCCATCTCTTCCCGCCCCCTC
CGTCCCCCTGCACGGACTCATCCTGACCTCGGCCGGGCCACTCTGGCTTCTCTGT
GCCCCTGTAAATAGTTTTAAATATGAACAAAGAAAAAAATATATTTTA
TGATTTAAAAAATAAATATAATTGGGATTTTAAAAACATGAGAAATGT
GAACTGTGATGGGGTGGGCAGGGCTGGGAGAACTTTGTACAGTGGAACAAA
TATTTATAAACTTAATTTTGTAAAACAG

Figure 14

GGCTGGTCTTGAACTCCTGGCCTGAGATGATCCTCTCTCCTCGGAAAGTGCTG
GGATTATAGCCTCGCCGCTCCTGCTATTTGCCAACCGCCGGGACGTACGGCTGG
TGGACGCCGGCGGAGTCAAGCTGGAGTCCACCATCGTGGTCAGCGGCCTGGAG
GATGCGGCCGCAGTGGACTTCCAGTTTTCCAAGGGAGCCGTGTACTGGACAG
ACGGAGCGAGGAGGCCATCAAGCAGACCTACCTGAACCAGACGGGGGCCGCC
GTGCAGAACGTGGTCATCTCCGGCCTGGTCTCTCCCGACGGCCTCGCCTGCGACT
GGGTGGGCAAGAAGCTGTACTGGACGGACTCAGAGACCAACCGCATCGAGGT
GGCCAACCTCAATGGCACATCCCGGAAGGTGCTCTTCTGGCAGGACCTTGACC
AGCCGAGGGCCATCGCCTTGGACCCCGCTCACGGGTACATGTACTGGACAGAC
TGGGGTGAGACGCCCCGGATTGAGCGGGCAGGGATGGATGGCAGCACCCGGA
AGATCATTGTGGACTCGGACATTTACTGGCCCAATGGACTGACCATCGACCT
GGAGGAGCAGAAGCTCTACTGGGCTGACGCCAAGCTCAGCTTCATCCACCGTG
CCAACCTGGACGGCTCGTTCCGGCAGAAGGTGGTGGAGGGCAGCCTGACGCAC
CCCTTCGCCCTGACGCTCTCCGGGGACACTCTGTACTGGACAGACTGGCAGACC
CGCTCCATCCATGCCTGCAACAAGCGCACTGGGGGGAAGAGGAAGGAGATCC
TGAGTGCCCTCTACTCACCCATGGACATCCAGGTGCTGAGCCAGGAGCGGCAG
CCTTTCTTCCACACTCGCTGTGAGGAGGACAATGGCGGCTGCTCCCACCTGTGC
CTGCTGTCCCCAAGCGAGCCTTTCTACACATGCGCCTGCCCCACGGGTGTGCAG
CTGCAGGACAACGGCAGGACGTGTAAGGCAGGAGCCGAGGAGGTGCTGCTGC
TGGCCCGGCGGACGGACCTACGGAGGATCTCGCTGGACACGCCGGACTTTACCG
ACATCGTGCTGCAGGTGGACGACATCCGGCACGCCATTGCCATCGACTACGAC
CCGCTAGAGGGCTATGTCTACTGGACAGATGACGAGGTGCGGGCCATCCGCAG
GGCGTACCTGGACGGGTCTGGGGCGCAGACGCTGGTCAACACCGAGATCAACG
ACCCCGATGGCATCGCGGTCGACTGGGTGGCCCGAAACCTCTACTGGACCGAC
ACGGGCACGGACCGCATCGAGGTGACGCGCCTCAACGGCACCTCCCGCAAGAT
CCTGGTGTCGGAGGACCTGGACGAGCCCCGAGCCATCGCACTGCACCCCGTGAT
GGGCCTCATGTACTGGACAGACTGGGGAGAGAACCCTAAAATCGAGTGTGCC
AACTTGGATGGGCAGGAGCGGCGTGTGCTGGTCAATGCCTCCCTCGGGTGGCCC
AACGGCCTGGCCCTGGACCTGCAGGAGGGGAAGCTCTACTGGGGAGACGCCA
AGACAGACAAGATCGAGGTGATCAATGTTGATGGGACGAAGAGGCGGACC
CTCCTGGAGGACAAGCTCCCGCACATTTTCGGGTTCACGCTGCTGGGGGACTTC
ATCTACTGGACTGACTGGCAGCGCCGCAGCATCGAGCGGGTGCACAAGGTCA
AGGCCAGCCGGGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAA
GCTGTGAATGTGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACG
GGGGGTGCAGCCACCTGTGCTTCTTCACACCCACGCAACCCGGTGTGGCTGCCC
CATCGGCCT
GGAGCTGCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCTTCTTGGTCT
TCACCAGCAGAGCCGCCATCCACAGGATCTCCCTCGAGACCAATAACAACGA
CGTGGCCATCCCGCTCACGGGCGTCAAGGAGGCCTCAGCCCTGGACTTTGATGT

Figure 14, ctd,

GTCCAACAACCACATCTACTGGACAGACGTCAGCCTGAAGACCATCAGCCGC
GCCTTCATGAACGGGAGCTCGGTGGAGCACGTGGTGGAGTTTGGCCTTGACTA
CCCCGAGGGCATGGCCGTTGACTGGATGGGCAAGAACCTCTACTGGGCCGACA
CTGGGACCAACAGAATCGAAGTGGCGCGGCTGGACGGGCAGTTCCGGCAAGT
CCTCGTGTGGAGGGACTTGGACAACCCGAGGTCGCTGGCCCTGGATCCCACCA
AGGGCTACATCTACTGGACCGAGTGGGGCGGCAAGCCGAGGATCGTGCGGGCC
TTCATGGACGGGACCAACTGCATGA
CGCTGGTGGACAAGGTGGGCCGGGCCAACGACCTCACCATTGACTACGCTGAC
CAGCGCCTCTACTGGACCGACCTGGACACCAACATGATCGAGTCGTCCAACA
TGCTGGGTCAGGAGCGGGTCGTGATTGCCGACGATCTCCCGCACCCGTTCGGTC
TGACGCAGTACAGCGATTATATCTACTGGACAGACTGGAATCTGCACAGCA
TTGAGCGGGCCGACAAGACTAGCGGCCGGAACCGCACCCTCATCCAGGGCCAC
CTGGACTTCGTGATGGACATCCTGGTGTTCCACTCCTCCCGCCAGGATGGCCTC
AATGACTGTATGCACAACAACGGGCAGTGTGGGCAGCTGTGCCTTGCCATCC
CCGGCGGCCACCGCTGCGGCTGCGCCTCACACTACACCCTGGACCCCAGCAGCCG
CAACTGCAGCCCGCCCACCACCTTCTTGCTGTTCAGCCAGAAATCTGCCATCA
GTCGGATGATCCCGGACGACCAGCACAGCCCGGATCTCATCCTGCCCCTGCATG
GACTGAGGAACGTCAAAGCCATCGACTATGACCCACTGGACAAGTTCATCT
ACTGGGTGGATGGGCGCCAGAACATCAAGCGAGCCAAGGACGACGGGACCCA
GCCCTTTGTTTTGACCTCTCTGAGCCAAGGCCAAAACCCAGACAGGCAGCCCC
ACGACCTCAGCATCGACATCTACAGCCGGACACTGTTCTGGACGTGCGAGGCC
ACCAATACCATCAACGTCCACAGGCTGAGCGGGGAAGCCATGGGGGTGGTGC
TGCGTGGGGACCGCGACAAGCCCAGGGCCATCGTCGTCAACGCGGAGCGAGGG
TACCTGTACTTCACCAACATGCAGGACCGGGCAGCCAAGATCGAACGCGCAG
CCCTGGACGGCACCGAGCGCGAGGTCCTCTTCACCACCGGCCTCATCCGCCCTGT
GGCCCTGGTGGTAGACAACACACTGGGCAAGCTGTTCTGGGTGGACGC
GGACCTGAAGCGCATTGAGAGCTGTGACCTGTCAGGGGCCAACCGCCTGACCC
TGGAGGACGCCAACATCGTGCAGCCTCTGGGCCTGACCATCCTTGGCAAGCAT
CTCTACTGGATCGACCGCCAGCAGCAGATGATCGAGCGTGTGGAGAAGACCA
CCGGGGACAAGCGGACTCGCATCCAGGGCCGTGTCGCCCACCTCACTGGCATCC
ATGCAGTGGAGGAAGTCAGCCTGGAGGAGTTCTCAGCCCACCCATGTGCCCGT
GACAATGGTGGCTGCTCCCACATCTGTATTGCCAAGGGTGATGGGACACCAC
GGTGCTCATGCCCAGTCCACCTCGTGCTCCTGCAGAACCTGCTGACCTGTGGAG
AGCCGCCCACCTGCTCCCCGGACCAGTTTGCATGTGCCACAGGGGAGATCGACT
GTATCCCCGGGGCCTGGCGCTGTGACGGCTTTCCCGAGTGCGATGACCAGAGCG
ACGAGGAGGGCTGCCCCGTGTGCTCCGCCGCCCAGTTCCCCTGCGCGCGGGGTCA
GTGTGTGGACCTGCGCCTGCGCTGCGACGGCGAGGCAGACTGTCAGGACCGCTC
AGACGAGGCGGACTGTGACGCCATCTGCCTGCCCAACCAGTTCCGGTGTGCGA
GCGGCCAGTGTGTCCTCATCAAACAGCAGTGCGACTCCTTCCCCGACTGTATC
GACGGCTCCGACGAGCTCATGTGTGAAATCACCAAGCCGCCCTCAGACGACA
GCCCGGCCCACAGCAGTGCCATCGGGCCCGTCATTGGCATCATCCTCTCTCTCTT

Figure 14, ctd.

CGTCATGGGTGGTGTCTATTTTGTGTGCCAGCGCGTGGTGTGCCAGCGCTATGC
GGGGGCCAACGGGCCCTTCCCGCACGAGTATGTCAGCGGGACCCCGCACGTGCC
CCTCAATTTCATAGCCCCGGGCGGTTCCCAGCATGGCCCCTTCACAGGCATCGC
ATGCGGAAAGTCCATGATGAGCTCCGTGAGCCTGATGGGGGGCCGGGGCGGGG
TGCCCCTCTACGACCGGAACCACGTCACAGGGGCCTCGTCCAGCAGCTCGTCCA
GCACGAAGGCCACGCTGTACCCGCCGATCCTGAACCCGCCGCCCTCCCCGGCCAC
GGACCCCTCCCTGTACAACATGGACATGTTCTACTCTTCAAACATTCCGGCCA
CTGTGAGACCGTACAGGCCCTACATCATTCGAGGAATGGCGCCCCCGACGACG
CCCTGCAGCACCGACGTGTGTGACAGCGACTACAGCGCCAGCCGCTGGAAGGC
CAGCAAGTACTACCTGGATTTGAACTCGGACTCAGACCCCTATCCACCCCCAC
CCACGCCCCACAGCCAGTACCTGTCGGCGGAGGACAGCTGCCCGCCCTCGCCCGC
CACCGAGAGGAGCTACTTCCATCTCTTCCCGCCCCCTCCGTCCCCCTGCACGGAC
TCATCCTGACCTCGGCCGGGCCACTCTGGCTTCTCTGTGCCCCTGTAAATAGTT
TTAAATATGAACAAAGAAAAAAATATATTTTATGATTTAAAAAATAA
ATATAATTGGGATTTTAAAAACATGAGAAATGTGAACTGTGATGGGGTG
GGCAGGGCTGGGAGAACTTTGTACAGTGGAACAAATATTTATAAACTTAA
TTTTGTAAAACAG

Figure 15(a)

AGGCTGGTCTCAAACTCCTGGCCTTAAGTGATCTGCCCGCCTCGGCCTCCCAAA
GTGCTGAGATGACAGGTGTGAGCCACCGTGCCCGGCCCAGAACTCTTTAATTC
CCACCTGAAACTTGCCGCCTTAAGCAGGTCCCCAGTCTCCCTCCCCTAGTCCCT
GGTCCCACCATTCTGCTTTCTGTCTCAATGAATTTGCCTACCCCTCGCCGCTCCT
GCTATTTGCCAACCGCCGGGACGTACGGCTGGTGGACGCCGGCGGAGTCAAGC
TGGAGTCCACCATCGTGGTCAGCGGCCTGGAGGATGCGGCCGCAGTGGACTTCC
AGTTTTCCAAGGGAGCCGTGTACTGGACAGACGTGAGCGAGGAGGCCATCA
AGCAGACCTACCTGAACCAGACGGGGGCCGCCGTGCAGAACGTGGTCATCTCC
GGCCTGGTCTCTCCCGACGGCCTCGCCTGCGACTGGGTGGGCAAGAAGCTGTAC
TGGACGGACTCAGAGACCAACCGCATCGAGGTGGCCAACCTCAATGGCACAT
CCCGGAAGGTGCTCTTCTGGCAGGACCTTGACCAGCCGAGGGCCATCGCCTTGG
ACCCCGCTCACGGGTACATGTACTGGACAGACTGGGGTGAGACGCCCCGGATT
GAGCGGGCAGGGATGGATGGCAGCACCCGGAAGATCATTGTGGACTCGGACA
TTTACTGGCCCAATGGACTGACCATCGACCTGGAGGAGCAGAAGCTCTACTG
GGCTGACGCCAAGCTCAGCTTCATCCACCGTGCCAACCTGGACGGCTCGTTCCG
GCAGAAGGTGGTGGAGGGCAGCCTGACGCACCCCTTCGCCCTGACGCTCTCCGG
GGACACTCTGTACTGGACAGACTGGCAGACCCGCTCCATCCATGCCTGCAACA
AGCGCACTGGGGGGAAGAGGAAGGAGATCCTGAGTGCCCTCTACTCACCCAT
GGACATCCAGGTGCTGAGCCAGGAGCGGCAGCCTTTCTTCCACACTCGCTGTG
AGGAGGACAATGGCGGCTGCTCCCACCTGTGCCTGCTGTCCCCAAGCGAGCCTT
TCTACACATGCGCCTGCCCCACGGGTGTGCAGCTGCAGGACAACGGCAGGACG
TGTAAGGCAGGAGCCGAGGAGGTGCTGCTGCTGGCCCGGCGGACGGACCTACG
GAGGATCTCGCTGGACACGCCGGACTTTACCGACATCGTGCTGCAGGTGGACG
ACATCCGGCACGCCATTGCCATCGACTACGACCCGCTAGAGGGCTATGTCTAC
TGGACAGATGACGAGGTGCGGGCCATCCGCAGGGCGTACCTGGACGGGTCTGG
GGCGCAGACGCTGGTCAACACCGAGATCAACGACCCCGATGGCATCGCGGTCG
ACTGGGTGGCCCGAAACCTCTACTGGACCGACACGGGCACGGACCGCATCGAG
GTGACGCGCCTCAACGGCACCTCCCGCAAGATCCTGGTGTCGGAGGACCTGGA
CGAGCCCCGAGCCATCGCACTGCACCCCGTGATGGGCCTCATGTACTGGACAG
ACTGGGGAGAGAACCCTAAAATCGAGTGTGCCAACTTGGATGGGCAGGAGC
GGCGTGTGCTGGTCAATGCCTCCCTCGGGTGGCCCAACGGCCTGGCCCTGGACCT
GCAGGAGGGGAAGCTCTACTGGGGAGACGCCAAGACAGACAAGATCGAGGT
GATCAATGTTGATGGGACGAAGAGGCGGACCCTCCTGGAGGACAAGCTCCCG
CACATTTTCGGGTTCACGCTGCTGGGGGACTTCATCTACTGGACTGACTGGCA
GCGCCGCAGCATCGAGCGGGTGCACAAGGTCAAGGCCAGCCGGGACGTCATCA
TTGACCAGCTGCCCGACCTGATGGGGCTCAAAGCTGTGAATGTGGCCAAGGTC
GTCGGAACCAACCCGTGTGCGGACAGGAACGGGGGGTGCAGCCACCTGTGCTT
CTTCACACCCCACGCAACCCGGTGTGGCTGCCCCATCGGCCTGGAGCTGCTGAG
TGACATGAAGACCTGCATCGTGCCTGAGGCCTTCTTGGTCTTCACCAGCAGAG

Figure 15(a), ctd.

CCGCCATCCACAGGATCTCCCTCGAGACCAATAACAACGACGTGGCCATCCCG
CTCACGGGCGTCAAGGAGGCCTCAGCCCTGGACTTTGATGTGTCCAACAACCA
CATCTACTGGACAGACGTCAGCCTGAAGACCATCAGCCGCGCCTTCATGAAC
GGGAGCTCGGTGGAGCACGTGGTGGAGTTTGGCCTTGACTACCCCGAGGGCAT
GGCCGTTGACTGGATGGGCAAGAACCTCTACTGGGCCGACACTGGGACCAAC
AGAATCGAAGTGGCGCGGCTGGACGGGCAGTTCCGGCAAGTCCTCGTGTGGAG
GGACTTGGACAACCCGAGGTCGCTGGCCCTGGATCCCACCAAGGGCTACATCT
ACTGGACCGAGTGGGGCGGCAAGCCGAGGATCGTGCGGGCCTTCATGGACGGG
ACCAACTGCATGACGCTGGTGGACAAGGTGGGCCGGGCCAACGACCTCACCA
TTGACTACGCTGACCAGCGCCTCTACTGGACCGACCTGGACACCAACATGATC
GAGTCGTCCAACATGCTGGGTCAGGAGCGGGTCGTGATTGCCGACGATCTCCC
GCACCCGTTCGGTCTGACGCAGTACAGCGATTATATCTACTGGACAGACTGG
AATCTGCACAGCATTGAGCGGGCCGACAAGACTAGCGGCCGGAACCGCACCC
TCATCCAGGGCCACCTGGACTTCGTGATGGACATCCTGGTGTTCCACTCCTCCC
GCCAGGATGGCCTCAATGACTGTATGCACAACAACGGGCAGTGTGGGCAGCT
GTGCCTTGCCATCCCCGGCGGCCACCGCTGCGGCTGCGCCTCACACTACACCCTG
GACCCCAGCAGCCGCAACTGCAGCCCGCCCACCACCTTCTTGCTGTTCAGCCAG
AAATCTGCCATCAGTCGGATGATCCCGGACGACCAGCACAGCCCGGATCTCA
TCCTGCCCCTGCATGGACTGAGGAACGTCAAAGCCATCGACTATGACCCACTG
GACAAGTTCATCTACTGGGTGGATGGGCGCCAGAACATCAAGCGAGCCAAG
GACGACGGGACCCAGCCCTTTGTTTTGACCTCTCTGAGCCAAGGCCAAAACCC
AGACAGGCAGCCCCACGACCTCAGCATCGACATCTACAGCCGGACACTGTTCT
GGACGTGCGAGGCCACCAATACCATCAACGTCCACAGGCTGAGCGGGGAAGC
CATGGGGGTGGTGCTGCGTGGGGACCGCGACAAGCCCAGGGCCATCGTCGTCA
ACGCGGAGCGAGGGTACCTGTACTTCACCAACATGCAGGACCGGGCAGCCAA
GATCGAACGCGCAGCCCTGGACGGCACCGAGCGCGAGGTCCTCTTCACCACCGG
CCTCATCCGCCCTGTGGCCCTGGTGGTAGACAACACACTGGGCAAGCTGTTCTG
GGTGGACGCGGACCTGAAGCGCATTGAGAGCTGTGACCTGTCAGGGGCCAACC
GCCTGACCCTGGAGGACGCCAACATCGTGCAGCCTCTGGGCCTGACCATCCTTG
GCAAGCATCTCTACTGGATCGACCGCCAGCAGCAGATGATCGAGCGTGTGGA
GAAGACCACCGGGGACAAGCGGACTCGCATCCAGGGCCGTGTCGCCCACCTCA
CTGGCATCCATGCAGTGGAGGAAGTCAGCCTGGAGGAGTTCTCAGCCCACCCA
TGTGCCCGTGACAATGGTGGCTGCTCCCACATCTGTATTGCCAAGGGTGATGG
GACACCACGGTGCTCATGCCCAGTCCACCTCGTGCTCCTGCAGAACCTGCTGAC
CTGTGGAGAGCCGCCCACCTGCTCCCCGGACCAGTTTGCATGTGCCACAGGGGA
GATCGACTGTATCCCCGGGGCCTGGCGCTGTGACGGCTTTCCCGAGTGCGATGA
CCAGAGCGACGAGGAGGGCTGCCCCGTGTGCTCCGCCGCCCAGTTCCCCTGCGCG
CGGGGTCAGTGTGTGGACCTGCGCCTGCGCTGCGACGGCGAGGCAGACTGTCAG
GACCGCTCAGACGAGGCGGACTGTGACGCCATCTGCCTGCCCAACCAGTTCCGG
TGTGCGAGCGGCCAGTGTGTCCTCATCAAACAGCAGTGCGACTCCTTCCCCGA
CTGTATCGACGGCTCCGACGAGCTCATGTGTGAAATCACCAAGCCGCCCTCAG

Figure 15(a), ctd.

ACGACAGCCCGGCCCACAGCAGTGCCATCGGGCCCGTCATTGGCATCATCCTCT
CTCTCTTCGTCATGGGTGGTGTCTATTTTGTGTGCCAGCGCGTGGTGTGCCAGC
GCTATGCGGGGGCCAACGGGCCCTTCCCGCACGAGTATGTCAGCGGGACCCCGC
ACGTGCCCCTCAATTTCATAGCCCCGGGCGGTTCCCAGCATGGCCCCTTCACAG
GCATCGCATGCGGAAAGTCCATGATGAGCTCCGTGAGCCTGATGGGGGGCCGG
GGCGGGGTGCCCCTCTACGACCGGAACCACGTCACAGGGGCCTCGTCCAGCAGC
TCGTCCAGCACGAAGGCCACGCTGTACCCGCGGATCCTGAACCCGCCGCCCTCCC
CGGCCACGGACCCCTCCCTGTACAACATGGACATGTTCTACTCTTCAAACATT
CCGGCCACTGCGAGACCGTACAGGCCCTACATCATTCGAGGAATGGCGCCCCC
GACGACGCCCTGCAGCACCGACGTGTGTGACAGCGACTACAGCGCCAGCCGCT
GGAAGGCCAGCAAGTACTACCTGGATTTGAACTCGGACTCAGACCCCTATCC
ACCCCCACCCACGCCCCACAGCCAGTACCTGTCGGCGGAGGACAGCTGCCCGCCC
TCGCCCGCCACCGAGAGGAGCTACTTCCATCTCTTCCCGCCCCCTCCGTCCCCCTG
CACGGACTCATCCTGACCTCGGCCGGGCCACTCTGGCTTCTCTGTGCCCCTGTAA
ATAGTTTTAAATATGAACAAAGAAAAAAATATATTTTATGATTTAAA
AAATAAATATAATTGGGATTTTAAAAACATGAGAAATGTGAACTGTGA
TGGGGTGGGCAGGGCTGGGAGAACTTTGTACAGTGGAACAAATATTTATA
AACTTAATTTTGTAAAACAG

Figure 15(b)

CAATGTCCAGTTCCGCTGCAGTTATAACATCCCATTTTTTGATTTCTTTTTA
TTTTTTCCTTTTTCTTTTTGAGATGGAGTCTCGCTCTGTCACCCAGGCTGGAGT
GCAATGGG

Figure 16(a)

GCCGCGGCGCCCGAGGCGGGAGCAAGAGGCGCCGGGAGCCGCGAGGATCCACCG
CCGCCGCGCGCGCCATGGAGCCCGAGTGAGCGCGCGGCGCTCCCGGCCGCCGGAC
GACATGGAAACGGCGCCGACCCGGGCCCCTCCGCCGCCGCCGCCGCCGCTGCTGC
TGCTGGTGCTGTACTGCAGCTTGGTCCCCGCCGCGGCCTCACCGCTCCTGTTGTT
TGCCAACCGCCGGGATGTGCGGCTAGTGGATGCCGGCGGAGTGAAGCTGGAGT
CCACCATTGTGGCCAGTGGCCTGGAGGATGCAGCTGCTGTAGACTTCCAGTTC
TCCAAGGGTGCTGTGTACTGGACAGATGTGAGCGAGGAGGCCATCAAACAG
ACCTACCTGAACCAGACTGGAGGTGCTGCACAGAACATTGTCATCTCGGGCC
TCGTGTCACCTGATGGCCTGGCCTGTGACTGGGTTGGCAAGAAGCTGTACTGG
ACGGACTCCGAGACCAACCGCATTGAGGTTGCCAACCTCAATGGGACGTCCCG
TAAGGTTCTCTTCTGGCAGGACCTGGACCAGCCAAGGGCCATTGCCCTGGATC
CTGCACATGGGTACATGTACTGGACTGACTGGGGGGAAGCACCCCGGATCGA
GCGGGCAGGGATGGATGGCAGTACCCGGAAGATCATTGTAGACTCCGACATT
TACTGGCCCAATGGGCTGACCATCGACCTGGAGGAACAGAAGCTGTACTGGG
CCGATGCCAAGCTCAGCTTCATCCACCGTGCCAACCTGGACGGCTCCTTCCGGC
AGAAGGTGGTGGAGGGCAGCCTCACTCACCCTTTTGCCCTGACACTCTCTGGG
GACACACTCTACTGGACAGACTGGCAGACCCGCTCCATCCACGCCTGCAACA
AGTGGACAGGGGAGCAGAGGAAGGAGATCCTTAGTGCTCTGTACTCACCCA
TGGACATCCAAGTGCTGAGCCAGGAGCGGCAGCCTCCCTTCCACACACCATGC
GAGGAGGACAACGGTGGCTGTTCCCACCTGTGCCTGCTGTCCCCGAGGGAGCCT
TTCTACTCCTGTGCCTGCCCCACTGGTGTGCAGTTGCAGGACAATGGCAAGAC
GTGCAAGACAGGGGCTGAGGAAGTGCTGCTGCTGGCTCGGAGGACAGACCTG
AGGAGGATCTCTCTGGACACCCCTGACTTCACAGACATAGTGCTGCAGGTGG
GCGACATCCGGCATGCCATTGCCATTGACTACGATCCCCTGGAGGGCTACGTG
TACTGGACCGATGATGAGGTGCGGGCTATCCGCAGGGCGTACCTAGATGGCTC
AGGTGCGCAGACACTTGTGAACACTGAGATCAATGACCCCGATGGCATTGCT
GTGGACTGGGTCGCCCCGGAACCTCTACTGGACAGATACAGGCACTGACAGAA
TTGAGGTGACTCGCCTCAACGGCACCTCCCGAAAGATCCTGGTATCTGAGGAC
CTGGACGAACCGCGAGCCATTGTGTTGCACCCTGTGATGGGCCTCATGTACTG
GACAGACTGGGGGGAGAACCCCAAAATCGAATGCGCCAACCTAGATGGGAG
AGATCGGCATGTCCTGGTGAACACCTCCCTTGGGTGGCCCAATGGACTGGCCC
TGGACCTGCAGGAGGGCAAGCTGTACTGGGGGGATGCCAAAACTGATAAAA
TCGAGGTGATCAACATAGACGGG

Figure 16(b)

GCCGCGGCGCCCGAGGCGGGAGCAAGAGGCGCCGGGAGCCGCGAGGATCCACCG
CCGCCGCGCGCGCCATGGAGCCCGAGTGAGCGCGCGGCGCTCCCGGCCGCCGGAC
GACATGGAAACGGCGCCGACCCGGCCCCTCCGCCGCCGCCGCCGCCGCTGCTGC
TGCTGGTGCTGTACTGCAGCTTGGTCCCCGCCGCGG

Figure 16(c)

ATGGAAACGGCGCCGACCCGGGCCCCTCCGCCGCCGCCGCCGCCGCTGCTGCTGCT
GGTGCTGTACTGCAGCTTGGTCCCCGCCGCGGCCTCACCGCTCCTGTTGTTTGCC.
AACCGCCGGGATGTGCGGCTAGTGGATGCCGGCGGAGTGAAGCTGGAGTCCAC
CATTGTGGCCAGTGGCCTGGAGGATGCAGCTGCTGTAGACTTCCAGTTCTCCA
AGGGTGCTGTGTACTGGACAGATGTGAGCGAGGAGGCCATCAAACAGACCT
ACCTGAACCAGACTGGAGGTGCTGCACAGAACATTGTCATCTCGGGCCTCGT
GTCACCTGATGGCCTGGCCTGTGACTGGGTTGGCAAGAAGCTGTACTGGACGG
ACTCCGAGACCAACCGCATTGAGGTTGCCAACCTCAATGGGACGTCCCGTAA
GGTTCTCTTCTGGCAGGACCTGGACCAGCCAAGGGCCATTGCCCTGGATCCTGC
ACATGGGTACATGTACTGGACTGACTGGGGGGGAAGCACCCCGGATCGAGCGG
GCAGGGATGGATGGCAGTACCCGGAAGATCATTGTAGACTCCGACATTTAC
TGGCCCAATGGGCTGACCATCGACCTGGAGGAACAGAAGCTGTACTGGGCCG
ATGCCAAGCTCAGCTTCATCCACCGTGCCAACCTGGACGGCTCCTTCCGGCAG
AAGGTGGTGGAGGGCAGCCTCACTCACCCTTTTGCCCTGACACTCTCTGGGGA
CACACTCTACTGGACAGACTGGCAGACCCGCTCCATCCACGCCTGCAACAAGT
GGACAGGGGAGCAGAGGAAGGAGATCCTTAGTGCTCTGTACTCACCCATGG
ACATCCAAGTGCTGAGCCAGGAGCGGCAGCCTCCCTTCCACACACCATGCGAG
GAGGACAACGGTGGCTGTTCCCACCTGTGCCTGCTGTCCCCGAGGGAGCCTTTC
TACTCCTGTGCCTGCCCCACTGGTGTGCAGTTGCAGGACAATGGCAAGACGTG
CAAGACAGGGGCTGAGGAAGTGCTGCTGCTGGCTCGGAGGACAGACCTGAGG
AGGATCTCTCTGGACACCCCTGACTTCACAGACATAGTGCTGCAGGTGGGCG
ACATCCGGCATGCCATTGCCATTGACTACGATCCCCTGGAGGGCTACGTGTAC
TGGACCGATGATGAGGTGCGGGCTATCCGCAGGGCGTACCTAGATGGCTCAGG
TGCGCAGACACTTGTGAACACTGAGATCAATGACCCCGATGGCATTGCTGTG
GACTGGGTCGCCCGGAACCTCTACTGGACAGATACAGGCACTGACAGAATTG
AGGTGACTCGCCTCAACGGCACCTCCCGAAAGATCCTGGTATCTGAGGACCTG
GACGAACCGCGAGCCATTGTGTTGCACCCTGTGATGGGCCTCATGTACTGGAC
AGACTGGGGGGGAGAACCCCAAAATCGAATGCGCCAACCTAGATGGGAGAG
ATCGGCATGTCCTGGTGAACACCTCCCTTGGGTGGCCCAATGGACTGGCCCTGG
ACCTGCAGGAGGGCAAGCTGTACTGGGGGGATGCCAAAACTGATAAAATCG
AGGTGATCAACATAGACGGG

Figure 16(d)

METAPTRAPPPPPPPLLLLVLYCSLVPAAASPLLLFANRRDVRLVDAGGVK
LESTIVASGLEDAAAVDFQFSKGAVYWTDVSEEAIKQTYLNQTGGAAQNIVI
SGLVSPDGLACDWVGKKLYWTDSETNRIEVANLNGTSRKVLFWQDLDQPRA
IALDPAHGYMYWTDWGEAPRIERAGMDGSTRKIIVDSDIYWPNGLTIDLEE
QKLYWADAKLSFIHRANLDGSFRQKVVEGSLTHPFALTLSGDTLYWTDWQT
RSIHACNKWTGEQRKEILSALYSPMDIQVLSQERQPPFHTPCEEDNGGCSHLCL
LSPREPFYSCACPTGVQLQDNGKTCKTGAEEVLLLARRTDLRRISLDTPDFTDI
VLQVGDIRHAIAIDYDPLEGYVYWTDDEVRAIRRAYLDGSGAQTLVNTEIND
PDGIAVDWVARNLYWTDTGTDRIEVTRLNGTSRKILVSEDLDEPRAIVLHP
VMGLMYWTDWGENPKIECANLDGRDRHVLVNTSLGWPNGLALDLQEGKLY
WGDAKTDKIEVINIDG

Figure 17(a)

CCTCGCCGCTCCTGCTATTTGCCAACCGCCGGGACGTACGGCTGGTGGACGCCGG
CGGAGTCAAGCTGGAGTCCACCATCGTGGTCAGCGGCCTGGAGGATGCGGCCG
CAGTGGACTTCCAGTTTTCCAAGGGAGCCGTGTACTGGACAGACGTGAGCGA
GGAGGCCATCAAGCAGACCTACCTGAACCAGACGGGGGCCGCCGTGCAGAAC
GTGGTCATCTCCGGCCTGGTCTCTCCCGACGGCCTCGCCTGCGACTGGGTGGGCA
AGAAGCTGTACTGGACGGACTCAGAGACCAACCGCATCGAGGTGGCCAACCT
CAATGGCACATCCCGGAAGGTGCTCTTCTGGCAGGACCTTGACCAGCCGAGGG
CCATCGCCTTGGACCCCGCTCACGGGTACATGTACTGGACAGACTGGGGTGAG
ACGCCCCGGATTGAGCGGGCAGGGATGGATGGCAGCACCCGGAAGATCATTG
TGGACTCGGACATTTACTGGCCCAATGGACTGACCATCGACCTGGAGGAGCA
GAAGCTCTACTGGGCTGACGCCAAGCTCAGCTTCATCCACCGTGCCAACCTGG
ACGGCTCGTTCCGGCAGAAGGTGGTGGAGGGCAGCCTGACGCACCCCTTCGCCC
TGACGCTCTCCGGGGACACTCTGTACTGGACAGACTGGCAGACCCGCTCCATCC
ATGCCTGCAACAAGCGCACTGGGGGGAAGAGGAAGGAGATCCTGAGTGCCCT
CTACTCACCCATGGACATCCAGGTGCTGAGCCAGGAGCGGCAGCCTTTCTTCC
ACACTCGCTGTGAGGAGGACAATGGCGGCTGCTCCCACCTGTGCCTGCTGTCCC
CAAGCGAGCCTTTCTACACATGCGCCTGCCCCACGGGTGTGCAGCTGCAGGAC
AACGGCAGGACGTGTAAGGCAGGAGCCGAGGAGGTGCTGCTGCTGGCCCGGCG
GACGGACCTACGGAGGATCTCGCTGGACACGCCGGACTTTACCGACATCGTGC
TGCAGGTGGACGACATCCGGCACGCCATTGCCATCGACTACGACCCGCTAGAG
GGCTATGTCTACTGGACAGATGACGAGGTGCCGGGCCATCCGCAGGGCGTACCT
GGACGGGTCTGGGGCGCAGACGCTGGTCAACACCGAGATCAACGACCCCGATG
GCATCGCGGTCGACTGGGTGGCCCGAAACCTCTACTGGACCGACACGGGCACG
GACCGCATCGAGGTGACGCGCCTCAACGGCACCTCCCGCAAGATCCTGGTGTCG
GAGGACCTGGACGAGCCCCGAGCCATCGCACTGCACCCCGTGATGGGCCTCATG
TACTGGACAGACTGGGGAGAGAACCCTAAAATCGAGTGTGCCAACTTGGAT
GGGCAGGAGCGGCGTGTGCTGGTCAATGCCTCCCTCGGGTGGCCCAACGGCCTG
GCCCTGGACCTGCAGGAGGGGAAGCTCTACTGGGGAGACGCCAAGACAGACA
AGATCGAGGTGATCAATGTTGATGGGACGAAGAGGCGGACCCTCCTGGAGG
ACAAGCTCCCGCACATTTTCGGGTTCACGCTGCTGGGGGACTTCATCTACTGG
ACTGACTGGCAGCGCCGCAGCATCGAGCGGGTGCACAAGGTCAAGGCCAGCCG
GGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAAGCTGTGAAT
GTGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACGGGGGGTGCA
GCCACCTGTGCTTCTTCACACCCCACGCAACCCGGTGTGGCTGCCCCATCGGCCT
GGAGCTGCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCTTCTTGGTCT
TCACCAGCAGAGCCGCCATCCACAGGATCTCCCTCGAGACCAATAACAACGA
CGTGGCCATCCCGCTCACGGGCGTCAAGGAGGCCTCAGCCCTGGACTTTGATGT
GTCCAACAACCACATCTACTGGACAGACGTCAGCCTGAAGACCATCAGCCGC
GCCTTCATGAACGGGAGCTCGGTGGAGCACGTGGTGGAGTTTGGCCTTGACTA
CCCCGAGGGCATGGCCGTTGACTGGATGGGCAAGAACCTCTACTGGGCCGACA

Figure 17(a) Continued

CTGGGACCAACAGAATCGAAGTGGCGCGGCTGGACGGGCAGTTCCGGCAAGT
CCTCGTGTGGAGGGACTTGGACAACCCGAGGTCGCTGGCCCTGGATCCCACCA
AGGGCTACATCTACTGGACCGAGTGGGGCGGCAAGCCGAGGATCGTGCGGGCC
TTCATGGACGGGACCAACTGCATGACGCTGGTGGACAAGGTGGGCCGGGCCA
ACGACCTCACCATTGACTACGCTGACCAGCGCCTCTACTGGACCGACCTGGAC
ACCAACATGATCGAGTCGTCCAACATGCTGGGTCAGGAGCGGGTCGTGATTG
CCGACGATCTCCCGCACCCGTTCGGTCTGACGCAGTACAGCGATTATATCTAC
TGGACAGACTGGAATCTGCACAGCATTGAGCGGGCCGACAAGACTAGCGGCC
GGAACCGCACCCTCATCCAGGGCCACCTGGACTTCGTGATGGACATCCTGGTG
TTCCACTCCTCCCGCCAGGATGGCCTCAATGACTGTATGCACAACAACGGGCA
GTGTGGGCAGCTGTGCCTTGCCATCCCCGGCGGCCACCGCTGCGGCTGCGCCTCA
CACTACACCCTGGACCCCAGCAGCCGCAACTGCAGCCCGCCCACCACCTTCTTG
CTGTTCAGCCAGAAATCTGCCATCAGTCGGATGATCCCGGACGACCAGCACA
GCCCGGATCTCATCCTGCCCCTGCATGGACTGAGGAACGTCAAAGCCATCGAC
TATGACCCACTGGACAAGTTCATCTACTGGGTGGATGGGCGCCAGAACATCA
AGCGAGCCAAGGACGACGGGACCCAGCCCTTTGTTTTGACCTCTCTGAGCCAA
GGCCAAAACCCAGACAGGCAGCCCCACGACCTCAGCATCGACATCTACAGCC
GGACACTGTTCTGGACGTGCGAGGCCACCAATACCATCAACGTCCACAGGCT
GAGCGGGGAAGCCATGGGGGTGGTGCTGCGTGGGGACCGCGACAAGCCCAGGG
CCATCGTCGTCAACGCGGAGCGAGGGTACCTGTACTTCACCAACATGCAGGA
CCGGGCAGCCAAGATCGAACGCGCAGCCCTGGACGGCACCGAGCGCGAGGTCC
TCTTCACCACCGGCCTCATCCGCCCTGTGGCCCTGGTGGTAGACAACACACTGG
GCAAGCTGTTCTGGGTGGACGCGGACCTGAAGCGCATTGAGAGCTGTGACCTG
TCAGGGGCCAACCGCCTGACCCTGGAGGACGCCAACATCGTGCAGCCTCTGGGC
CTGACCATCCTTGGCAAGCATCTCTACTGGATCGACCGCCAGCAGCAGATGA
TCGAGCGTGTGGAGAAGACCACCGGGGACAAGCGGACTCGCATCCAGGGCCG
TGTCGCCCACCTCACTGGCATCCATGCAGTGGAGGAAGTCAGCCTGGAGGAGT
TCTCAGCCCACCCATGTGCCCGTGACAATGGTGGCTGCTCCCACATCTGTATTG
CCAAGGGTGATGGGACACCACGGTGCTCATGCCCAGTCCACCTCGTGCTCCTGC
AGAACCTGCTGACCTGTGGAGAGCCGCCCACCTGCTCCCCGGACCAGTTTGCAT
GTGCCACAGGGGAGATCGACTGTATCCCCGGGGCCTGGCGCTGTGACGGCTTTC
CCGAGTGCGATGACCAGAGCGACGAGGAGGGCTGCCCCGTGTGCTCCGCCGCCC
AGTTCCCCTGCGCGCGGGGTCAGTGTGTGGACCTGCGCCTGCGCTGCGACGGCGA
GGCAGACTGTCAGGACCGCTCAGACGAGGCGGACTGTGACGCCATCTGCCTGC
CCAACCAGTTCCGGTGTGCGAGCGGCCAGTGTGTCCTCATCAAACAGCAGTGC
GACTCCTTCCCCGACTGTATCGACGGCTCCGACGAGCTCATGTGTGAAATCAC
CAAGCCGCCCTCAGACGACAGCCCGGCCCACAGCAGTGCCATCGGGCCCGTCAT
TGGCATCATCCTCTCTCTCTTCGTCATGGGTGGTGTCTATTTTGTGTGCCAGCG
CGTGGTGTGCCAGCGCTATGCGGGGGCCAACGGGCCCTTCCCGCACGAGTATGT
CAGCGGGACCCCGCACGTGCCCCTCAATTTCATAGCCCCGGGCGGTTCCCAGCA
TGGCCCCTTCACAGGCATCGCATGCGGAAAGTCCATGATGAGCTCCGTGAGCC

Figure 17(a) Continued

TGATGGGGGGCCGGGGCGGGGTGCCCCTCTACGACCGGAACCACGTCACAGGGG
CCTCGTCCAGCAGCTCGTCCAGCACGAAGGCCACGCTGTACCCGCCGATCCTGA
ACCCGCCGCCCTCCCCGGCCACGGACCCCTCCCTGTACAACATGGACATGTTCT
ACTCTTCAAACATTCCGGCCACTGTGAGACCGTACAGGCCCTACATCATTCG
AGGAATGGCGCCCCCGACGACGCCCTGCAGCACCGACGTGTGTGACAGCGACT
ACAGCGCCAGCCGCTGGAAGGCCAGCAAGTACTACCTGGATTTGAACTCGGA
CTCAGACCCCTATCCACCCCCACCCACGCCCCACAGCCAGTACCTGTCGGCGGA
GGACAGCTGCCCGCCCTCGCCCGCCACCGAGAGGAGCTACTTCCATCTCTTCCCG
CCCCCTCCGTCCCCCTGCACGGACTCATCCTGACCTCGGCCGGGCCACTCTGGCTT
CTCTGTGCCCCTGTAAATAGTTTTAAATATGAACAAAGAAAAAAATATA
TTTTATGATTTAAAAAATAAATATAATTGGGATTTTAAAAACATGAGA
AATGTGAACTGTGATGGGGTGGGCAGGGCTGGGAGAACTTTGTACAGTGGA
ACAAATATTTATAAACTTAATTTTGTAAAACAG

Figure 17(b)

SPLLLFANRRDVRLVDAGGVKLESTIV
VSGLEDAAAVDFQFSKGAVYWTDVSEEAIKQTYLNQTGAAVQNVVISGLVSPDGLAC
DWVGKKLYWTDSETNRIEVANLNGTSRKVLFWQDLDQPRAIALDPAHGYMYWTDW
GETPRIERAGMDGSTRKIIVDSDIYWPNGLTIDLEEQKLYWADAKLSFIHRANLDGSFR
QKVVEGSLTHPFALTLSGDTLYWTDWQTRSIHACNKRTGGKRKEILSALYSPMDIQVLS
QERQPFFHTRCEEDNGGCSHLCLLSPSEPFYTCACPTGVQLQDNGRTCKAGAEEVLLL
ARRTDLRRISLDTPDFTDIVLQVDDIRHAIAIDYDPLEGYVYWTDDEVRAIRRAYLDGS
GAQTLVNTEINDPDGIAVDWVARNLYWTDTGTDRIEVTRLNGTSRKILVSEDLDEPRAI
ALHPVMGLMYWTDWGENPKIECANLDGQERRVLVNASLGWPNGLALDLQEGKLYW
GDAKTDKIEVINVDGTKRRTLLEDKLPHIFGFTLLGDFIYWTDWQRRSIERVHKVKASR
DVIIDQLPDLMGLKAVNVAKVVGTNPCADRNGGCSHLCFFTPHATRCGCPIGLELLSD
MKTCIVPEAFLVFTSRAAIHRISLETNNNDVAIPLTGVKEASALDFDVSNNHIYWTDVSL
KTISRAFMNGSSVEHVVEFGLDYPEGMAVDWMGKNLYWADTGTNRIEVARLDGQFR
QVLVWRDLDNPRSLALDPTKGYIYWTEWGGKPRIVRAFMDGTNCMTLVDKVGRAND
LTIDYADQRLYWTDLDTNMIESSNMLGQERVVIADDLPHPFGLTQYSDYIYWTDWNL
HSIERADKTSGRNRTLIQGHLDFVMDILVFHSSRQDGLNDCMHNNGQCGQLCLAIPGG
HRCGCASHYTLDPSSRNCSPPTTFLLFSQKSAISRMIPDDQHSPDLILPLHGLRNVKAIDY
DPLDKFIYWVDGRQNIKRAKDDGTQPFVLTSLSQGQNPDRQPHDLSIDIYSRTLFWTCE
ATNTINVHRLSGEAMGVVLRGDRDKPRAIVVNAERGYLYFTNMQDRAAKIERAALDG
TEREVLFTTGLIRPVALVVDNTLGKLFWVDADLKRIESCDLSGANRLTLEDANIVQPLG
LTILGKHLYWIDRQQQMIERVEKTTGDKRTRIQGRVAHLTGIHAVEEVSLEEFSAHPCA
RDNGGCSHICIAKGDGTPRCSCPVHLVLLQNLLTCGEPPTCSPDQFACATGEIDCIPGA
WRCDGFPECDDQSDEEGCPVCSAAQFPCARGQCVDLRLRCDGEADCQDRSDEADCD
AICLPNQFRCASGQCVLIKQQCDSFPDCIDGSDELMCEITKPPSDDSPAHSSAIGPVIGIIL
SLFVMGGVYFVCQRVVCQRYAGANGPFPHEYVSGTPHVPLNFIAPGGSQHGPFTGIAC
GKSMMSSVSLMGGRGGVPLYDRNHVTGASSSSSSSTKATLYPPILNPPPSPATDPSLYN
MDMFYSSNIPATVRPYRPYIIRGMAPPTTPCSTDVCDSDYSASRWKASKYYLDLNSDSD
PYPPPPTPHSQYLSAEDSCPPSPATERSYFHLFPPPPSPCTDSS

Figure 18(a)

GCCGCGGCGCCCGAGGCGGGAGCAAGAGGCGCCGGGAGCCGCGAGGATCCACCGCCGCCG
CGCGCGCCATGGAGCCCGAGTGAGCGCGCGGCGCTCCCGGCCGCCGGACGACATGGAAAC
GGCGCCGACCCGGGCCCCTCCGCCGCCGCCGCCGCCGCTGCTGCTGCTGGTGCTGTACTG
CAGCTTGGTCCCCGCCGCGGCCTCACCGCTCCTGTTGTTTGCCAACCGCCGGGATGTGCG
GCTAGTGGATGCCGGCGGAGTGAAGCTGGAGTCCACCATTGTGGCCAGTGGCCTGGAGGA
TGCAGCTGCTGTAGACTTCCAGTTCTCCAAGGGTGCTGTGTACTGGACAGATGTGAGCGA
GGAGGCCATCAAACAGACCTACCTGAACCAGACTGGAGCTGCTGCACAGAACATTGTCAT
CTCGGGCCTCGTGTCACCTGATGGCCTGGCCTGTGACTGGGTTGGCAAGAAGCTGTACTG
GACGGACTCCGAGACCAACCGCATTGAGGTTGCCAACCTCAATGGGACGTCCCGTAAGGT
TCTCTTCTGGCAGGACCTGGACCAGCCAAGGGCCATTGCCCTGGATCCTGCACATGGGTA
CATGTACTGGACTGACTGGGGGGAAGCACCCCGGATCGAGCGGGCAGGGATGGATGGCAG
TACCCGGAAGATCATTGTAGACTCCGACATTTACTGGCCCAATGGGCTGACCATCGACCT
GGAGGAACAGAAGCTGTACTGGGCCGATGCCAAGCTCAGCTTCATCCACCGTGCCAACCT
GGACGGCTCCTTCCGGCAGAAGGTGGTGGAGGGCAGCCTCACTCACCCTTTTGCCCTGAC
ACTCTCTGGGGACACACTCTACTGGACAGACTGGCAGACCCGCTCCATCCACGCCTGCAA
CAAGTGGACAGGGGAGCAGAGGAAGGAGATCCTTAGTGCTCTGTACTCACCCATGGACAT
CCAAGTGCTGAGCCAGGAGCGGCAGCCTCCCTTCCACACACCATGCGAGGAGGACAACGG
TGGCTGTTCCCACCTGTGCCTGCTGTCCCCGAGGGAGCCTTTCTACTCCTGTGCCTGCCC
CACTGGTGTGCAGTTGCAGGACAATGGCAAGACGTGCAAGACAGGGGCTGAGGAAGTGCT
GCTGCTGGCTCGGAGGACAGACCTGAGGAGGATCTCTCTGGACACCCCTGACTTCACAGA
CATAGTGCTGCAGGTGGGCGACATCCGGCATGCCATTGCCATTGACTACGATCCCCTGGA
GGGCTACGTGTACTGGACCGATGATGAGGTGCGGGCTATCCGCAGGGCGTACCTAGATGG
CTCAGGTGCGCAGACACTTGTGAACACTGAGATCAATGACCCCGATGGCATTGCTGTGGA
CTGGGTCGCCCGGAACCTCTACTGGACAGATACAGGCACTGACAGAATTGAGGTGACTCG
CCTCAACGGCACCTCCCGAAAGATCCTGGTATCTGAGGACCTGGACGAACCGCGAGCCAT
TGTGTTGCACCCTGTGATGGGCCTCATGTACTGGACAGACTGGGGGGAGAACCCCAAAAT
CGAATGCGCCAACCTAGATGGGAGAGATCGGCATGTCCTGGTGAACACCTCCCTTGGGTG
GCCCAATGGACTGGCCCTGGACCTGCAGGAGGGCAAGCTGTACTGGGGGGATGCCAAAAC
TGATAAAATCGAGGTGATCAACATAGACGGGACAAAGCGGAAGACCCTGCTTGAGGACAA
GCTCCCACACATTTTTGGGTTCACACTGCTGGGGGACTTCATCTACTGGACCGACTGGCA
GAGACGCAGTATTGAAAGGGTCCACAAGGTCAAGGCCAGCCGGGATGTCATCATTGATCA
ACTCCCCCGACCTGATGGGACTCAAAGCCGTGAATGTGGCCAAGGTTGTCGGAACCAACCC
ATGTGCGGATGGAAATGGAGGGTGCAGCCATCTGTGCTTCTTCACCCCACGTGCCACCAA
GTGTGGCTGCCCCATTGGCCTGGAGCTGTTGAGTGACATGAAGACCTGCATAATCCCCGA
GGCCTTCCGGTATTCACCAGCAGAGCCACCATCCACAGGATCTCCCTGGAGACTAACAAC
AACGATGTGGCTATCCCACTCACGGGTGTCAAAGAGGCCTCTGCACTGGACTTTGATGTG
TCCAACAATCACATCTACTGGACTGATGTTAGCCTCAAGACGATCAGCCGAGCCTTCATG
AATGGGAGCTCAGTGGAGCACGTGATTGAGTTTGGCCTCGACTACCCTGAAGGAATGGCT
GTGGACTGGATGGGCAAGAACCTCTATTGGGCGGACACAGGGACCAACAGGATTGAGGTG
GCCCGGCTGGATGGGCAGTTCCGGCAGGTGCTTGTGTGGAGAGACCTTGACAACCCCAGG
TCTCTGGCTCTGGATCCTACTAAAGGCTACATCTACTGGACTGAGTGGGGTGGCAAGCCA
AGGATTGTGCGGGCCTTCATGGATGGGACCAATTGTATGACACTGGTAGACAAGGTGGGC
CGGGCCAACGACCTCACCATTGATTATGCCGACCAGCGACTGTACTGGACTGACCTGGAC
ACCAACATGATTGAGTCTTCCAACATGCTGGGTCAGGAGCGCATGGTGATAGCTGACGAT
CTGCCCTACCCGTTTGGCCTGACTCAATATAGCGATTACATCTACTGGACTGACTGGAAC
CTGCATAGCATTGAACGGGCGGACAAGACCAGTGGGCGGAACCGCACCCTCATCCAGGGT
CACCTGGACTTCGTCATGGACATCCTGGTGTTCCACTCCTCCCGTCAGGATGGCCTCAAC

Figure 18(a) Continued

GACTGCGTGCACAGCAATGGCCAGTGTGGGCAGCTGTGCCTCGCCATCCCCGGAGGCCAC
CGCTGTGGCTGTGCTTCACACTACACGCTGGACCCCAGCAGCCGCAACTGCAGCCCGCCC
TCCACCTTCTTGCTGTTCAGCCAGAAATTTGCCATCAGCCGGATGATCCCCGATGACCAG
CTCAGCCCGGACCTTGTCCTACCCCTTCATGGGCTGAGGAACGTCAAAGCCATCAACTAT
GACCCGCTGGACAAGTTCATCTACTGGGTGGACGGGCGCCAGAACATCAAGAGGGCCAAG
GACGACGGTACCCAGCCCTCCATGCTGACCTCTCCCAGCCAAAGCCTGAGCCCAGACAGA
CAGCCACACGACCTCAGCATTGACATCTACAGCCGGACACTGTTCTGGACCTGTGAGGCC
ACCAACACTATCAATGTCCACCGGCTGGATGGGGATGCCATGGGAGTGGTGCTTCGAGGG
GACCGTGACAAGCCAAGGGCCATTGCTGTCAATGCTGAGCGAGGGTACATGTACTTTACC
AACATGCAGGACCATGCTGCCAAGATCGAGCGAGCCTCCCTGGATGGCACAGAGCGGGAG
GTCCTCTTCACCACAGGCCTCATCCGTCCCGTGGCCCTTGTGGTGGACAATGCTCTGGGC
AAGCTCTTCTGGGTGGATGCCGACCTAAAGCGAATCGAAAGCTGTGACCTCTCTCTGGGGCC
AACCGCCTGACCCTGGAAGATGCCAACATCGTACAGCCAGTAGGTCTGACAGTGCTGGGC
AGGCACCTCTACTGGATCGACCGCCAGCAGCAGATGATCGAGCGCGTGGAGAAGACCACT
GGGGACAAGCGGACTAGGGTTCAGGGCCGTGTCACCCACCTGACAGGCATCCATGCCGTG
GAGGAAGTCAGCCTGGAGGAGTTCTCAGCCCATCCTTGTGCCCGAGACAATGGCGGCTGC
TCCCACATCTGTATCGCCAAGGGTGATGGAACACCGCGCTGCTCGTGCCCTGTCCACCTG
GTGCTCCTGCAGAACCTGCTGACTTGTGGTGAGCCTCCTACCTGCTCCCCTGATCAGTTT
GCATGTACCACTGGTGAGATCGACTGCATCCCCGGAGCCTGGCGCTGTGACGGCTTCCCT
GAGTGTGCTGACCAGAGTGATGAAGAAGGCTGCCCAGTGTGCTCCGCCTCTCAGTTCCCC
TGCGCTCGAGGCCAGTGTGTGGACCTGCGGTTACGCTGCGACGGTGAGGCCGACTGCCAG
GATCGCTCTGATGAAGTAACTGCGATGCTGTCTGTCTGCCCAATCAGTTCCGGTGCACCA
GCGGCCAGTGTGTCCTCATCAAGCAACAGTGTGACTCCTTCCCCGACTGTGCTGATGGGT
CTGATGAGCTCATGTGTGAAATCAACAAGCCACCCTCTGATGACATCCCAGCCCACAGCA
GTGCCATTGGGCCCGTCATTGGTATCATCCTCTCCCTCTTCGTCATGGGCGGGGTCTACT
TTGTCTGCCAGCGTGTGATGTGCCAGCGCTACACAGGGGCCAGTGGGCCCTTTCCCCACG
AGTATGTTGGTGGAGCCCCTCATGTGCCTCTCAACTTCATAGCCCCAGGTGGCTCACAGC
ACGGTCCCTTCCCAGGCATCCCGTGCAGCAAGTCCGTGATGAGCTCCATGAGCCTGGTGG
GGGGGCGCGGCAGCGTGCCCCTCTATGACCGGAATCACGTCACTGGGGCCTCATCCAGCA
GCTCGTCCAGCACAAAGGCCACACTATATCCGCCGATCCTGAACCCACCCCCGTCCCCGG
CCACAGACCCCTCTCTCTACAACGTGGACGTGTTTTATTCTTCAGGCATCCCGGCCACCG
CTAGACCATACAGGCCCTACGTCATTCGAGGTATGGCACCCCCAACAACACCGTGCAGCA
CAGATGTGTGTGACAGTGACTACAGCATCAGTCGCTGGAAGAGCAGCAAATACTACCTGG
ACTTGAATTCGGACTCAGACCCCTACCCCCCCCCGCCCACCCCCCACAGCCAGTACCTAT
CTGCAGAGGACAGCTGCCCACCCTCACCAGGCACTGAGAGGAGTTACTGCCACCTCTTCC
CGCCCCCACCGTCCCCCTGCACGGACTCGTCCTGACCTCGGCCGTCCACCCGGCCCTGCT
GCCTCCCTGTAAATATTTTTAAATATGAACAAAGGAAAAATATATTTTATGATTTAAAAA
ATAAATATAATTGGGGTTTTTAACAAGTGAGAAATGTGAGCGGTGAAGGGGTGGGCAGGG
CTGGGAAACTTTTCTAG

Figure 18(b)

ATGGAAACGGCGCCGACCCGGGCCCCTCCGCCGCCGCCGCCGCCGCTGCTGCTGCTGGTG
CTGTACTGCAGCTTGGTCCCCGCCGCGGCCTCACCGCTCCTGTTGTTTGCCAACCGCCGG
GATGTGCGGCTAGTGGATGCCGGCGGAGTGAAGCTGGAGTCCACCATTGTGGCCAGTGGC
CTGGAGGATGCAGCTGCTGTAGACTTCCAGTTCTCCAAGGGTGCTGTGTACTGGACAGAT
GTGAGCGAGGAGGCCATCAAACAGACCTACCTGAACCAGACTGGAGCTGCTGCACAGAAC
ATTGTCATCTCGGGCCTCGTGTCACCTGATGGCCTGGCCTGTGACTGGGTTGGCAAGAAG
CTGTACTGGACGGACTCCGAGACCAACCGCATTGAGGTTGCCAACCTCAATGGGACGTCC
CGTAAGGTTCTCTTCTGGCAGGACCTGGACCAGCCAAGGGCCATTGCCCTGGATCCTGCA
CATGGGTACATGTACTGGACTGACTGGGGGGAAGCACCCCGGATCGAGCGGGCAGGGATG
GATGGCAGTACCCGGAAGATCATTGTAGACTCCGACATTTACTGGCCCAATGGGCTGACC
ATCGACCTGGAGGAACAGAAGCTGTACTGGGCCGATGCCAAGCTCAGCTTCATCCACCGT
GCCAACCTGGACGGCTCCTTCCGGCAGAAGGTGGTGGAGGGCAGCCTCACTCACCCTTTT
GCCCTGACACTCTCTGGGGACACACTCTACTGGACAGACTGGCAGACCCGCTCCATCCAC
GCCTGCAACAAGTGGACAGGGGAGCAGAGGAAGGAGATCCTTAGTGCTCTGTACTCACCC
ATGGACATCCAAGTGCTGAGCCAGGAGCGGCAGCCTCCCTTCCACACACCATGCGAGGAG
GACAACGGTGGCTGTTCCCACCTGTGCCTGCTGTCCCCGAGGGAGCCTTTCTACTCCTGT
GCCTGCCCCACTGGTGTGCAGTTGCAGGACAATGGCAAGACGTGCAAGACAGGGGCTGAG
GAAGTGCTGCTGCTGGCTCGGAGGACAGACCTGAGGAGGATCTCTCTGGACACCCCTGAC
TTCACAGACATAGTGCTGCAGGTGGGCGACATCCGGCATGCCATTGCCATTGACTACGAT
CCCCTGGAGGGCTACGTGTACTGGACCGATGATGAGGTGCGGGCTATCCGCAGGGCGTAC
CTAGATGGCTCAGGTGCGCAGACACTTGTGAACACTGAGATCAATGACCCCGATGGCATT
GCTGTGGACTGGGTCGCCCGGAACCTCTACTGGACAGATACAGGCACTGACAGAATTGAG
GTGACTCGCCTCAACGGCACCTCCCGAAAGATCCTGGTATCTGAGGACCTGGACGAACCG
CGAGCCATTGTGTTGCACCCTGTGATGGGCCTCATGTACTGGACAGACTGGGGGGAGAAC
CCCAAAATCGAATGCGCCAACCTAGATGGGAGAGATCGGCATGTCCTGGTGAACACCTCC
CTTGGGTGGCCCAATGGACTGGCCCTGGACCTGCAGGAGGGCAAGCTGTACTGGGGGGAT
GCCAAAACTGATAAAATCGAGGTGATCAACATAGACGGGACAAAGCGGAAGACCCTGCTT
GAGGACAAGCTCCCACACATTTTTGGGTTCACACTGCTGGGGGACTTCATCTACTGGACC
GACTGGCAGAGACGCAGTATTGAAAGGGTCCACAAGGTCAAGGCCAGCCGGGATGTCATC
ATTGATCAACTCCCCGACCTGATGGGACTCAAAGCCGTGAATGTGGCCAAGGTTGTCGGA
ACCAACCCATGTGCGGATGGAAATGGAGGGTGCAGCCATCTGTGCTTCTTCACCCCACGT
GCCACCAAGTGTGGCTGCCCCATTGGCCTGGAGCTGTTGAGTGACATGAAGACCTGCATA
ATCCCCGAGGCCTTCCTGGTATTCACCAGCAGAGCCACCATCCACAGGATCTCCCTGGAG
ACTAACAACAACGATGTGGCTATCCCACTCACGGGTGTCAAAGAGGCCTCTGCACTGGAC
TTTGATGTTCCAACAATCACATCTACTGGACTGATGTTAGCCTCAAGACGATCAGCCGAG
CCTTCATGAATGGGAGCTCAGTGGAGCACGTGATTGAGTTTGGCCTCGACTACCCTGAAG
GAATGGCTGTGGACTGGATGGGCAAGAACCTCTATTGGGCGGACACAGGGACCAACAGGA
TTGAGGTGGCCCGGCTGGATGGGCAGTTCCGGCAGGTGCTTGTGTGGAGAGACCTTGACA
ACCCCAGGTCTCTGGCTCTGGATCCTACTAAAGGCTACATCTACTGGACTGAGTGGGGTG
GCAAGCCAAGGATTGTGCGGGCCTTCATGGATGGGACCAATTGTATGACACTGGTAGACA
AGGTGGGCCGGGCCAACGACCTCACCATTGATTATGCCGACCAGCGACTGTACTGGACTG
ACCTGGACACCAACATGATTGAGTCTTCCAACATGCTGGGTCAGGAGCGCATGGTGATAG
CTGACGATCTGCCCTACCCGTTTGGCCTGACTCAATATAGCGATTACATCTACTGGACTG
ACTGGAACCTGCATAGCATTGAACGGGCGGACAAGACCAGTGGGCGGAACCGCACCCTCA
TCCAGGGTCACCTGGACTTCGTCATGGACATCCTGGTGTTCCACTCCTCCCGTCAGGATG
GCCTCAACGACTGCGTGCACAGCAATGGCCAGTGTGGGCAGCTGTGCCTCGCCATCCCCG
GAGGCCACCGCTGTGGCTGTGCTTCACACTACACGCTGGACCCCAGCAGCCGCAACTGCA

Figure 18(b) Continued

GCCCGCCCTCCACCTTCTTGCTGTTCAGCCAGAAATTTGCCATCAGCCGGATGATCCCCG
ATGACCAGCTCAGCCCGGACCTTGTCCTACCCCTTCATGGGCTGAGGAACGTCAAAGCCA
TCAACTATGACCCGCTGGACAAGTTCATCTACTGGGTGGACGGGCGCCAGAACATCAAGA
GGGCCAAGGACGACGGTACCCAGCCCTCCATGCTGACCTCTCCCAGCCAAAGCCTGAGCC
CAGACAGACAGCCACACGACCTCAGCATTGACATCTACAGCCGGACACTGTTCTGGACCT
GTGAGGCCACCAACACTATCAATGTCCACCGGCTGGATGGGGATGCCATGGGAGTGGTGC
TTCGAGGGGACCGTGACAAGCCAAGGGCCATTGCTGTCAATGCTGAGCGAGGGTACATGT
ACTTTACCAACATGCAGGACCATGCTGCCAAGATCGAGCGAGCCTCCCTGGATGGCACAG
AGCGGGAGGTCCTCTTCACCACAGGCCTCATCCGTCCCGTGGCCCTTGTGGTGGACAATG
CTCTGGGCAAGCTCTTCTGGGTGGATGCCGACCTAAAGCGAATCGAAAGCTGTGACCTCT
CTGGGGCCAACCGCCTGACCCTGGAAGATGCCAACATCGTACAGCCAGTAGGTCTGACAG
TGCTGGGCAGGCACCTCTACTGGATCGACCGCCAGCAGCAGATGATCGAGCGCGTGGAGA
AGACCACTGGGGACAAGCGGACTAGGGTTCAGGGCCGTGTCACCCACCTGACAGGCATCC
ATGCCGTGGAGGAAGTCAGCCTGGAGGAGTTCTCAGCCCATCCTTGTGCCCGAGACAATG
GCGGCTGCTCCCACATCTGTATCGCCAAGGGTGATGGAACACCGCGCTGCTCGTGCCCTG
TCCACCTGGTGCTCCTGCAGAACCTGCTGACTTGTGGTGAGCCTCCTACCTGCTCCCCTG
ATCAGTTTGCATGTACCACTGGTGAGATCGACTGCATCCCCGGAGCCTGGCGCTGTGACG
GCTTCCCTGAGTGTGCTGACCAGAGTGATGAAGAAGGCTGCCCAGTGTGCTCCGCCTCTC
AGTTCCCCTGCGCTCGAGGCCAGTGTGTGGACCTGCGGTTACGCTGCGACGGTGAGGCCG
ACTGCCAGGATCGCTCTGATGAAGCTAACTGCGATGCTGTCTGTCTGCCCAATCAGTTCC
GGTGCACCAGCGGCCAGTGTGTCCTCATCAAGCAACAGTGTGACTCCTTCCCCGACTGTG
CTGATGGGTCTGATGACTCATGTGTGAAATCAACAAGCCACCCTCTGATGACATCCCAGC
CCACAGCAGTGCCATTGGGCCCGTCATTGGTATCATCCTCTCCCTCTTCGTCATGGGCGG
GGTCTACTTTGTCTGCCAGCGTGTGATGTGCCAGCGCTACACAGGGGCCAGTGGGCCCTT
TCCCCACGAGTATGTTGGTGGAGCCCCTCATGTGCCTCTCAACTTCATAGCCCCAGGTGG
CTCACAGCACGGTCCCTTCCCAGGCATCCCGTGCAGCAAGTCCGTGATGAGCTCCATGAG
CCTGGTGGGGGGGCGCGGCAGCGTGCCCCTCTATGACCGGAATCACGTCACTGGGGCCTC
ATCCAGCAGCTCGTCCAGCACAAAGGCCACACTATATCCGCCGATCCTGAACCCACCCCC
GTCCCCGGCCACAGACCCCTCTCTCTACAACGTGGACGTGTTTTATTCTTCAGGCATCCC
GGCCACCGCTAGACCATACAGGCCCTACGTCATTCGAGGTATGGCACCCCCAACAACACC
GTGCAGCACAGATGTGTGTGACAGTGACTACAGCATCAGTCGCTGGAAGAGCAGCAAATA
CTACCTGGACTTGAATTCGGACTCAGACCCCTACCCCCCCCCGCCCACCCCCCACAGCCA
GTACCTATCTGCAGAGGACAGCTGCCCACCCTCACCAGGCACTGAGAGGAGTTACTGCCA
CCTCTTCCCGCCCCCACCGTCCCCCTGCACGGACTCGTCCTGA

Figure 18(c)

```
   1  METAPTRAPP PPPPPLLLLV LYCSLVPAAA SPLLLFANRR DVRLVDAGGV
  51  KLESTIVASG LEDAAAVDFQ FSKGAVYWTD VSEEAIKQTY LNQTGAAAQN
 101  IVISGLVSPD GLACDWVGKK LYWTDSETNR IEVANLNGTS RKVLFWQDLD
 151  QPRAIALDPA HGYMYWTDWG EAPRIERAGM DGSTRKIIVD SDIYWPNGLT
 201  IDLEEQKLYW ADAKLSFIHR ANLDGSFRQK VVEGSLTHPF ALTLSGDTLY
 251  WTDWQTRSIH ACNKWTGEQR KEILSALYSP MDIQVLSQER QPPFHTPCEE
 301  DNGGCSHLCL LSPREPFYSC ACPTGVQLQD NGKTCKTGAE EVLLLARRTD
 351  LRRISLDTPD FTDIVLQVGD IRHAIAIDYD PLEGYVYWTD DEVRAIRRAY
 401  LDGSGAQTLV NTEINDPDGI AVDWVARNLY WTDTGTDRIE VTRLNGTSRK
 451  ILVSEDLDEP RAIVLHPVMG LMYWTDWGEN PKIECANLDG RDRHVLVNTS
 501  LGWPNGLALD LQEGKLYWGD AKTDKIEVIN IDGTKRKTLL EDKLPHIFGF
 551  TLLGDFIYWT DWQRRSIERV HKVKASRDVI IDQLPDLMGL KAVNVAKVVG
 601  TNPCADGNGG CSHLCFFTPR ATKCGCPIGL ELLSDMKTCI IPEAFLVFTS
 651  RATIHRISLE TNNNDVAIPL TGVKEASALD FDVSNNHIYW TDVSLKTISR
 701  AFMNGSSVEH VIEFGLDYPE GMAVDWMGKN LYWADTGTNR IEVARLDGQF
 751  RQVLVWRDLD NPRSLALDPT KGYIYWTEWG GKPRIVRAFM DGTNCMTLVD
 801  KVGRANDLTI DYADQRLYWT DLDTNMIESS NMLGQERMVI ADDLPYPFGL
 851  TQYSDYIYWT DWNLHSIERA DKTSGRNRTL IQGHLDFVMD ILVFHSSRQD
 901  GLNDCVHSNG QCGQLCLAIP GGHRCGCASH YTLDPSSRNC SPPSTFLLFS
 951  QKFAISRMIP DDQLSPDLVL PLHGLRNVKA INYDPLDKFI YWVDGRQNIK
1001  RAKDDGTQPS MLTSPSQSLS PDRQPHDLSI DIYSRTLFWT CEATNTINVH
1051  RLDGDAMGVV LRGDRDKPRA IAVNAERGYM YFTNMQDHAA KIERASLDGT
1101  EREVLFTTGL IRPVALVVDN ALGKLFWVDA DLKRIESCDL SGANRLTLED
1151  ANIVQPVGLT VLGRHLYWID RQQQMIERVE KTTGDKRTRV QGRVTHLTGI
1201  HAVEEVSLEE FSAHPCARDN GGCSHICIAK GDGTPRCSCP VHLVLLQNLL
1251  TCGEPPTCSP DQFACTTGEI DCIPGAWRCD GFPECADQSD EEGCPVCSAS
1301  QFPCARGQCV DLRLRCDGEA DCQDRSDEAN CDAVCLPNQF RCTSGQCVLI
1351  KQQCDSFPDC ADGSDELMCE INKPPSDDIP AHSSAIGPVI GIILSLFVMG
1401  GVYFVCQRVM CQRYTGASGP FPHEYVGGAP HVPLNFIAPG GSQHGPFPGI
1451  PCSKSVMSSM SLVGGRGSVP LYDRNHVTGA SSSSSSSTKA TLYPPILNPP
1501  PSPATDPSLY NVDVFYSSGI PATARPYRPY VIRGMAPPTT PCSTDVCDSD
1551  YSISRWKSSK YYLDLNSDSD PYPPPPTPHS QYLSAEDSCP PSPGTERSYC
1601  HLFPPPPSPC TDSS
```

Figure 18(d)

```
1 METAPTRAPPPPPPPLLLLVLYCSL.VPAAASPLLLFANRRDVRLVDAGG 49
  || ||   | |    ||||.  |    ||||||||||||||||||||||||
1 MEAAPPGPPWPLLLLLLLLLALCGCPAPAAASPLLLFANRRDVRLVDAGG 50

50 VKLESTIVASGLEDAAAVDFQFSKGAVYWTDVSEEAIKQTYLNQTGAAAQ 99
   |||||||| |||||||||||||||||||||||||||||||||||||||| |
51 VKLESTIVVSGLEDAAAVDFQFSKGAVYWTDVSEEAIKQTYLNQTGAAVQ 100

100 NIVISGLVSPDGLACDWVGKKLYWTDSETNRIEVANLNGTSRKVLFWQDL 149
    |:|||||||||||||||||||||||||||||||||||||||||||||||||
101 NVVISGLVSPDGLACDWVGKKLYWTDSETNRIEVANLNGTSRKVLFWQDL 150
.
150 DQPRAIALDPAHGYMYWTDWGEAPRIERAGMDGSTRKIIVDSDIYWPNGL 199
    |||||||||||||||||||| ||||||||||||||||||||||||||||
151 DQPRAIALDPAHGYMYWTDWGETPRIERAGMDGSTRKIIVDSDIYWPNGL 200

200 TIDLEEQKLYWADAKLSFIHRANLDGSFRQKVVEGSLTHPFALTLSGDTL 249
    ||||||||||||||||||||||||||||||||||||||||||||||||||
201 TIDLEEQKLYWADAKLSFIHRANLDGSFRQKVVEGSLTHPFALTLSGDTL 250

250 YWTDWQTRSIHACNKWTGEQRKEILSALYSPMDIQVLSQERQPPFHTPCE 299
    |||||||||||||||| || .|||||||||||||||||||| ||| ||
251 YWTDWQTRSIHACNKRTGGKRKEILSALYSPMDIQVLSQERQPFFHTRCE 300
.
300 EDNGGCSHLCLLSPREPFYSCACPTGVQLQDNGKTCKTGAEEVLLLARRT 349
    |||||||||||||| ||||.|||||||||||||:||| ||||||||||||
301 EDNGGCSHLCLLSPSEPFYTCACPTGVQLQDNGRTCKAGAEEVLLLARRT 350

350 DLRRISLDTPDFTDIVLQVGDIRHAIAIDYDPLEGYVYWTDDEVRAIRRA 399
    |||||||||||||||||| ||||||||||||||||||||||||||||||
351 DLRRISLDTPDFTDIVLQVDDIRHAIAIDYDPLEGYVYWTDDEVRAIRRA 400

400 YLDGSGAQTLVNTEINDPDGIAVDWVARNLYWTDTGTDRIEVTRLNGTSR 449
    ||||||||||||||||||||||||||||||||||||||||||||||||||
401 YLDGSGAQTLVNTEINDPDGIAVDWVARNLYWTDTGTDRIEVTRLNGTSR 450

450 KILVSEDLDEPRAIVLHPVMGLMYWTDWGENPKIECANLDGRDRHVLVNT 499
    |||||||||||||| |||||||||||||||||||||||.:| ||||
451 KILVSEDLDEPRAIALHPVMGLMYWTDWGENPKIECANLDGQERRVLVNA 500

500 SLGWPNGLALDLQEGKLYWGDAKTDKIEVINIDGTKRKTLLEDKLPHIFG 549
    |||||||||||||||||||||||||||||:||||||:||||||||||||
501 SLGWPNGLALDLQEGKLYWGDAKTDKIEVINVDGTKRRTLLEDKLPHIFG 550

550 FTLLGDFIYWTDWQRRSIERVHKVKASRDVIIDQLPDLMGLKAVNVAKVV 599
    ||||||||||||||||||||||||||||||||||||||||||||||||||
551 FTLLGDFIYWTDWQRRSIERVHKVKASRDVIIDQLPDLMGLKAVNVAKVV 600
```

Figure 18(d) Continued

```
600 GTNPCADGNGGCSHLCFFTPRATKCGCPIGLELLSDMKTCIIPEAFLVFT 649
    ||||||| |||||||||||| ||:||||||||||||||||||:||||||||
601 GTNPCADRNGGCSHLCFFTPHATRCGCPIGLELLSDMKTCIVPEAFLVFT 650

650 SRATIHRISLETNNNDVAIPLTGVKEASALDFDVSNNHIYWTDVSLKTIS 699
    ||| ||||||||||||||||||||||||||||||||||||||||||||||
651 SRAAIHRISLETNNNDVAIPLTGVKEASALDFDVSNNHIYWTDVSLKTIS 700

700 RAFMNGSSVEHVIEFGLDYPEGMAVDWMGKNLYWADTGTNRIEVARLDGQ 749
    ||||||||||||:||||||||||||||||||||||||||||||||||||
701 RAFMNGSSVEHVVEFGLDYPEGMAVDWMGKNLYWADTGTNRIEVARLDGQ 750

750 FRQVLVWRDLDNPRSLALDPTKGYIYWTEWGGKPRIVRAFMDGTNCMTLV 799
    ||||||||||||||||||||||||||||||||||||||||||||||||||
751 FRQVLVWRDLDNPRSLALDPTKGYIYWTEWGGKPRIVRAFMDGTNCMTLV 800

800 DKVGRANDLTIDYADQRLYWTDLDTNMIESSNMLGQERMVIADDLPYPFG 849
    ||||||||||||||||||||||||||||||||||||.||||||||:|||
801 DKVGRANDLTIDYADQRLYWTDLDTNMIESSNMLGQERVVIADDLPHPFG 850

850 LTQYSDYIYWTDWNLHSIERADKTSGRNRTLIQGHLDFVMDILVFHSSRQ 899
    ||||||||||||||||||||||||||||||||||||||||||||||||||
851 LTQYSDYIYWTDWNLHSIERADKTSGRNRTLIQGHLDFVMDILVFHSSRQ 900

900 DGLNDCVHSNGQCGQLCLAIPGGHRCGCASHYTLDPSSRNCSPPSTFLLF 949
    |||||||.|.|||||||||||||||||||||||||||||||||.|||||
901 DGLNDCMHNNGQCGQLCLAIPGGHRCGCASHYTLDPSSRNCSPPTTFLLF 950

950 SQKFAISRMIPDDQLSPDLVLPLHGLRNVKAINYDPLDKFIYWVDGRQNI 999
    ||| |||||||||| |||||:|||||||||||||.||||||||||||||||
951 SQKSAISRMIPDDQHSPDLILPLHGLRNVKAIDYDPLDKFIYWVDGRQNI 1000

1000 KRAKDDGTQPSMLTSPSQSLSPDRQPHDLSIDIYSRTLFWTCEATNTINV 1049
     |||||||||| .||| ||  .|||||||||||||||||||||||||||||
1001 KRAKDDGTQPFVLTSLSQGQNPDRQPHDLSIDIYSRTLFWTCEATNTINV 1050

1050 HRLDGDAMGVVLRGDRDKPRAIAVNAERGYMYFTNMQDHAAKIERASLDG 1099
     ||| |:|||||||||||||||| |||||||:|||||||| |||||||.|||
1051 HRLSGEAMGVVLRGDRDKPRAIVVNAERGYLYFTNMQDRAAKIERAALDG 1100

1100 TEREVLFTTGLIRPVALVVDNALGKLFWVDADLKRIESCDLSGANRLTLE 1149
     |||||||||||||||||||| ||||||||||||||||||||||||||||
1101 TEREVLFTTGLIRPVALVVDNTLGKLFWVDADLKRIESCDLSGANRLTLE 1150

1150 DANIVQPVGLTVLGRHLYWIDRQQQMIERVEKTTGDKRTRVQGRVTHLTG 1199
     |||||||.|||:||:|||||||||||||||||||||:|||| ||||
1151 DANIVQPLGLTILGKHLYWIDRQQQMIERVEKTTGDKRTRIQGRVAHLTG 1200
```

Figure 18(d) Continued

```
1200 IHAVEEVSLEEFSAHPCARDNGGCSHICIAKGDGTPRCSCPVHLVLLQNL 1249
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1201 IHAVEEVSLEEFSAHPCARDNGGCSHICIAKGDGTPRCSCPVHLVLLQNL 1250

1250 LTCGEPPTCSPDQFACTTGEIDCIPGAWRCDGFPECADQSDEEGCPVCSA 1299
     |||||||||||||||| |||||||||||||||||| ||||||||||||||
1251 LTCGEPPTCSPDQFACATGEIDCIPGAWRCDGFPECDDQSDEEGCPVCSA 1300

1300 SQFPCARGQCVDLRLRCDGEADCQDRSDEANCDAVCLPNQFRCTSGQCVL 1349
     .|||||||||||||||||||||||||||||.|||:|||||||| ||||||
1301 AQFPCARGQCVDLRLRCDGEADCQDRSDEADCDAICLPNQFRCASGQCVL 1350

1350 IKQQCDSFPDCADGSDELMCEINKPPSDDIPAHSSAIGPVIGIILSLFVM 1399
     |||||||||||| |||||||||| |||||| ||||||||||||||||||||
1351 IKQQCDSFPDCIDGSDELMCEITKPPSDDSPAHSSAIGPVIGIILSLFVM 1400

1400 GGVYFVCQRVMCQRYTGASGPFPHEYVGGAPHVPLNFIAPGGSQHGPFPG 1449
     ||||||||||.|||| ||.|||||||| | ||||||||||||||||| |
1401 GGVYFVCQRVVCQRYAGANGPFPHEYVSGTPHVPLNFIAPGGSQHGPFTG 1450

1450 IPCSKSVMSSMSLVGGRGSVPLYDRNHVTGASSSSSSSTKATLYPPILNP 1499
     | | ||.|||.||.|||| ||||||||||||||||||||||||||||||||
1451 IACGKSMMSSVSLMGGRGGVPLYDRNHVTGASSSSSSSTKATLYPPILNP 1500

1500 PPSPATDPSLYNVDVFYSSGIPATARPYRPYVIRGMAPPTTPCSTDVCDS 1549
     |||||||||||||.|.|.|||| |||| ||||||:||||||||||||||||
1501 PPSPATDPSLYNMDMFYSSNIPATVRPYRPYIIRGMAPPTTPCSTDVCDS 1550

1550 DYSISRWKSSKYYLDLNSDSDPYPPPPTPHSQYLSAEDSCPPSPGTERSY 1599
     ||| ||||.||||||||||||||||||||||||||||||||| |||||
1551 DYSASRWKASKYYLDLNSDSDPYPPPPTPHSQYLSAEDSCPPSPATERSY 1600

1600 CHLFPPPPSPCTDSS 1614
      |||||||||||||
1601 FHLFPPPPSPCTDSS 1615
```

Figure 18(e)

```
25 CPAPAAASPLLLFANRRDVRLVDAGGVKLESTIVVSGLEDAAAVDFQFSK 74
29 .....AASPLLLFANRRDVRLVDAGGVKLESTIVASGLEDAAAVDFQFSK 73

75 GAVYWTDVSEEAIKQTYLNQTGAAVQNVVISGLVSPDGLACDWVGKKLYW 124
74 GAVYWTDVSEEAIKQTYLNQTGAAAQNIVISGLVSPDGLACDWVGKKLYW 123

125 TDSETNRIEVANLNGTSRKVLFWQDLDQPRAIALDPAHGYMYWTDWGETP 174
124 TDSETNRIEVANLNGTSRKVLFWQDLDQPRAIALDPAHGYMYWTDWGEAP 173

175 RIERAGMDGSTRKIIVDSDIYWPNGLTIDLEEQKLYWADAKLSFIHRANL 224
174 RIERAGMDGSTRKIIVDSDIYWPNGLTIDLEEQKLYWADAKLSFIHRANL 223

225 DGSFRQKVVEGSLTHPFALTLSGDTLYWTDWQTRSIHACNKRTGGKRKEI 274
224 DGSFRQKVVEGSLTHPFALTLSGDTLYWTDWQTRSIHACNKWTGEQRKEI 273

275 LSALYSPMDIQVLSQERQPFFHTRCEEDNGGCSHLCLLSPSEPFYTCACP 324
274 LSALYSPMDIQVLSQERQPPFHTPCEEDNGGCSHLCLLSPREPFYSCACP 323

325 TGVQLQDNGRTCKAGAEEVLLLARRTDLRRISLDTPDFTDIVLQVDDIRH 374
324 TGVQLQDNGKTCKTGAEEVLLLARRTDLRRISLDTPDFTDIVLQVGDIRH 373

375 AIAIDYDPLEGYVYWTDDEVRAIRRAYLDGSGAQTLVNTEINDPDGIAVD 424
374 AIAIDYDPLEGYVYWTDDEVRAIRRAYLDGSGAQTLVNTEINDPDGIAVD 423

425 WVARNLYWTDTGTDRIEVTRLNGTSRKILVSEDLDEPRAIALHPVMGLMY 474
424 WVARNLYWTDTGTDRIEVTRLNGTSRKILVSEDLDEPRAIVLHPVMGLMY 473

475 WTDWGENPKIECANLDGQERRVLVNASLGWPNGLALDLQEGKLYWGDAKT 524
474 WTDWGENPKIECANLDGRDRHVLVNTSLGWPNGLALDLQEGKLYWGDAKT 523

525 DKIEVINVDGTKRRTLLEDKLPHIFGFTLLGDFIYWTDWQRRSIERVHKV 574
524 DKIEVINIDGTKRKTLLEDKLPHIFGFTLLGDFIYWTDWQRRSIERVHKV 573

575 KASRDVIIDQLPDLMGLKAVNVAKVVGTNPCADRNGGCSHLCFFTPHATR 624
574 KASRDVIIDQLPDLMGLKAVNVAKVVGTNPCADGNGGCSHLCFFTPRATK 623
```

Figure 18(e) Continued

```
625 CGCPIGLELLSDMKTCIVPEAFLVFTSRAAIHRISLETNNNDVAIPLTGV 674
    |||||||||||||||||:|||||||||||| |||||||||||||||||||||
624 CGCPIGLELLSDMKTCIIPEAFLVFTSRATIHRISLETNNNDVAIPLTGV 673

675 KEASALDFDVSNNHIYWTDVSLKTISRAFMNGSSVEHVVEFGLDYPEGMA 724
    |||||||||||||||||||||||||||||||||||||:||||||||||||
674 KEASALDFDVSNNHIYWTDVSLKTISRAFMNGSSVEHVIEFGLDYPEGMA 723

725 VDWMGKNLYWADTGTNRIEVARLDGQFRQVLVWRDLDNPRSLALDPTKGY 774
    |||||||||||||||||||||||||||||||||||||||||||||||||||
724 VDWMGKNLYWADTGTNRIEVARLDGQFRQVLVWRDLDNPRSLALDPTKGY 773

775 IYWTEWGGKPRIVRAFMDGTNCMTLVDKVGRANDLTIDYADQRLYWTDLD 824
    |||||||||||||||||||||||||||||||||||||||||||||||||||
774 IYWTEWGGKPRIVRAFMDGTNCMTLVDKVGRANDLTIDYADQRLYWTDLD 823

825 TNMIESSNMLGQERVVIADDLPHPFGLTQYSDYIYWTDWNLHSIERADKT 874
    |||||||||||||||.|||||||:|||||||||||||||||||||||||||
824 TNMIESSNMLGQERMVIADDLPYPFGLTQYSDYIYWTDWNLHSIERADKT 873

875 SGRNRTLIQGHLDFVMDILVFHSSRQDGLNDCMHNNGQCGQLCLAIPGGH 924
    |||||||||||||||||||||||||||||||.|.|||||||||||||||
874 SGRNRTLIQGHLDFVMDILVFHSSRQDGLNDCVHSNGQCGQLCLAIPGGH 923

925 RCGCASHYTLDPSSRNCSPPTTFLLFSQKSAISRMIPDDQHSPDLILPLH 974
    ||||||||||||||||||||.|||||||| |||||||||| ||||:||||
924 RCGCASHYTLDPSSRNCSPPSTFLLFSQKFAISRMIPDDQLSPDLVLPLH 973

975 GLRNVKAIDYDPLDKFIYWVDGRQNIKRAKDDGTQPFVLTSLSQGQNPDR 1024
    ||||||||.|||||||||||||||||||||||||| .||| || .|||
974 GLRNVKAINYDPLDKFIYWVDGRQNIKRAKDDGTQPSMLTSPSQSLSPDR 1023

1025 QPHDLSIDIYSRTLFWTCEATNTINVHRLSGEAMGVVLRGDRDKPRAIVV 1074
     |||||||||||||||||||||||||||| |:|||||||||||||||| |
1024 QPHDLSIDIYSRTLFWTCEATNTINVHRLDGDAMGVVLRGDRDKPRAIAV 1073

1075 NAERGYLYFTNMQDRAAKIERAALDGTEREVLFTTGLIRPVALVVDNTLG 1124
     |||||||:|||||||| |||||||.|||||||||||||||||||||| ||
1074 NAERGYMYFTNMQDHAAKIERASLDGTEREVLFTTGLIRPVALVVDNALG 1123

1125 KLFWVDADLKRIESCDLSGANRLTLEDANIVQPLGLTILGKHLYWIDRQQ 1174
     |||||||||||||||||||||||||||||||.|||:||:||||||||||
1124 KLFWVDADLKRIESCDLSGANRLTLEDANIVQPVGLTVLGRHLYWIDRQQ 1173

1175 QMIERVEKTTGDKRTRIQGRVAHLTGIHAVEEVSLEEFSAHPCARDNGGC 1224
     ||||||||||||||||:|||| |||||||||||||||||||||||||||||
1174 QMIERVEKTTGDKRTRVQGRVTHLTGIHAVEEVSLEEFSAHPCARDNGGC 1223
```

Figure 18(e) Continued

```
1225 SHICIAKGDGTPRCSCPVHLVLLQNLLTCGEPPTCSPDQFACATGEIDCI 1274
     ||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
1224 SHICIAKGDGTPRCSCPVHLVLLQNLLTCGEPPTCSPDQFACTTGEIDCI 1273

1275 PGAWRCDGFPECDDQSDEEGCPVCSAAQFPCARGQCVDLRLRCDGEADCQ 1324
     |||||||||||| |||||||||||||.|||||||||||||||||||||||
1274 PGAWRCDGFPECADQSDEEGCPVCSASQFPCARGQCVDLRLRCDGEADCQ 1323

1325 DRSDEADCDAICLPNQFRCASGQCVLIKQQCDSFPDCIDGSDELMCEITK 1374
     ||||||.|||:|||||||| |||||||||||||||| |||||||||| |
1324 DRSDEANCDAVCLPNQFRCTSGQCVLIKQQCDSFPDCADGSDELMCEINK 1373

1375 PPSDDSPAHSSAIGPVIGIILSLFVMGGVYFVCQRVVCQRYAGANGPFPH 1424
     ||||| |||||||||||||||||||||||||||||.|||| ||.|||||
1374 PPSDDIPAHSSAIGPVIGIILSLFVMGGVYFVCQRVMCQRYTGASGPFPH 1423

1425 EYVSGTPHVPLNFIAPGGSQHGPFTGIACGKSMMSSVSLMGGRGGVPLYD 1474
     ||| | |||||||||||||||||| || | ||.|||.||.|||| |||||
1424 EYVGGAPHVPLNFIAPGGSQHGPFPGIPCSKSVMSSMSLVGGRGSVPLYD 1473

1475 RNHVTGASSSSSSSTKATLYPPILNPPPSPATDPSLYNMDMFYSSNIPAT 1524
     |||||||||||||||||||||||||||||||||||||.|.|||| ||||
1474 RNHVTGASSSSSSSTKATLYPPILNPPPSPATDPSLYNVDVFYSSGIPAT 1523

1525 VRPYRPYIIRGMAPPTTPCSTDVCDSDYSASRWKASKYYLDLNSDSDPYP 1574
     ||||||:|||||||||||||||||||| |||||.|||||||||||||||
1524 ARPYRPYVIRGMAPPTTPCSTDVCDSDYSISRWKSSKYYLDLNSDSDPYP 1573

1575 PPPTPHSQYLSAEDSCPPSPATERSYFHLFPPPPSPCTDSS 1615
     |||||||||||||||||||| ||||| ||||||||||||||
1574 PPPTPHSQYLSAEDSCPPSPGTERSYCHLFPPPPSPCTDSS 1614
```

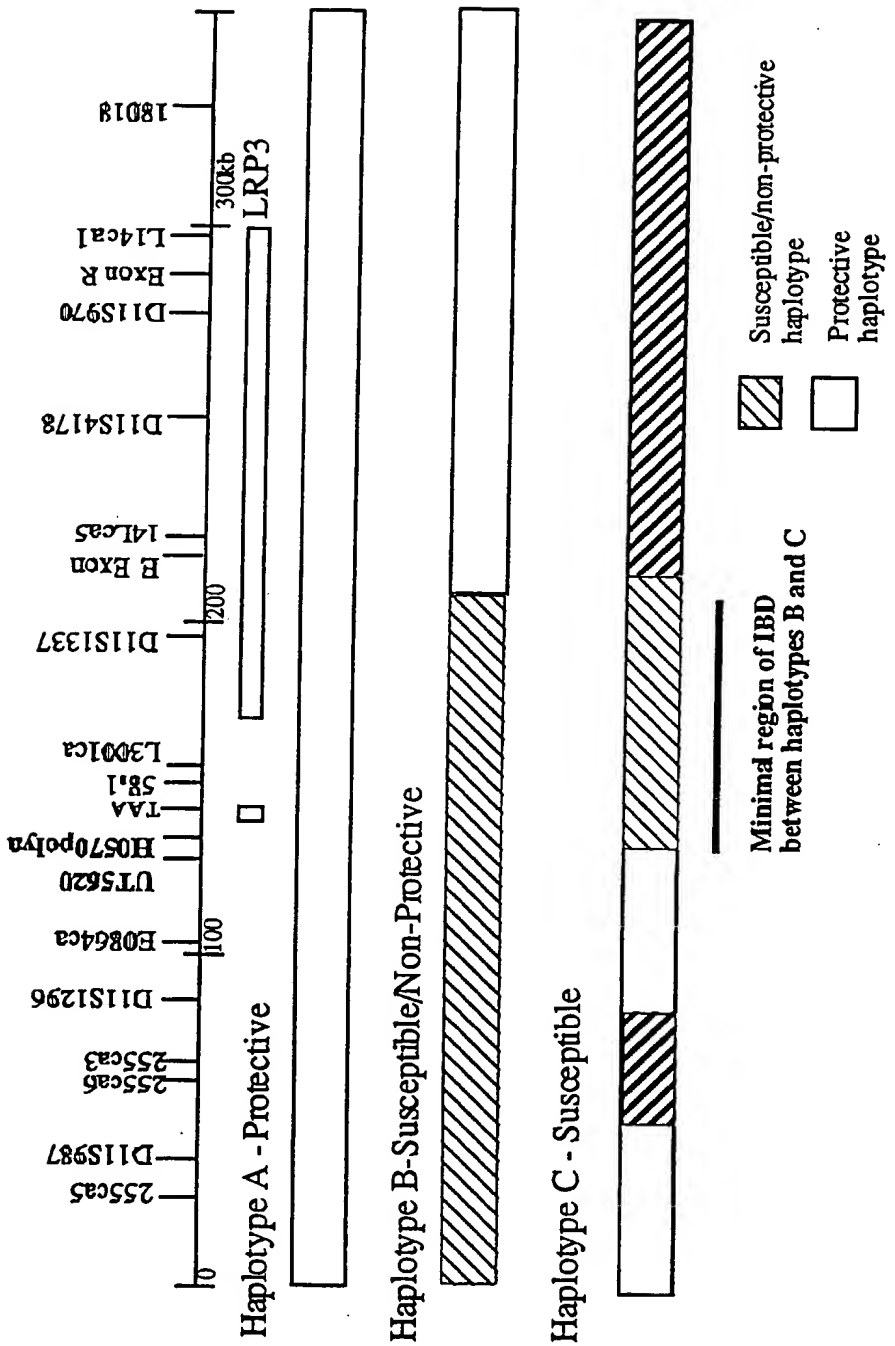
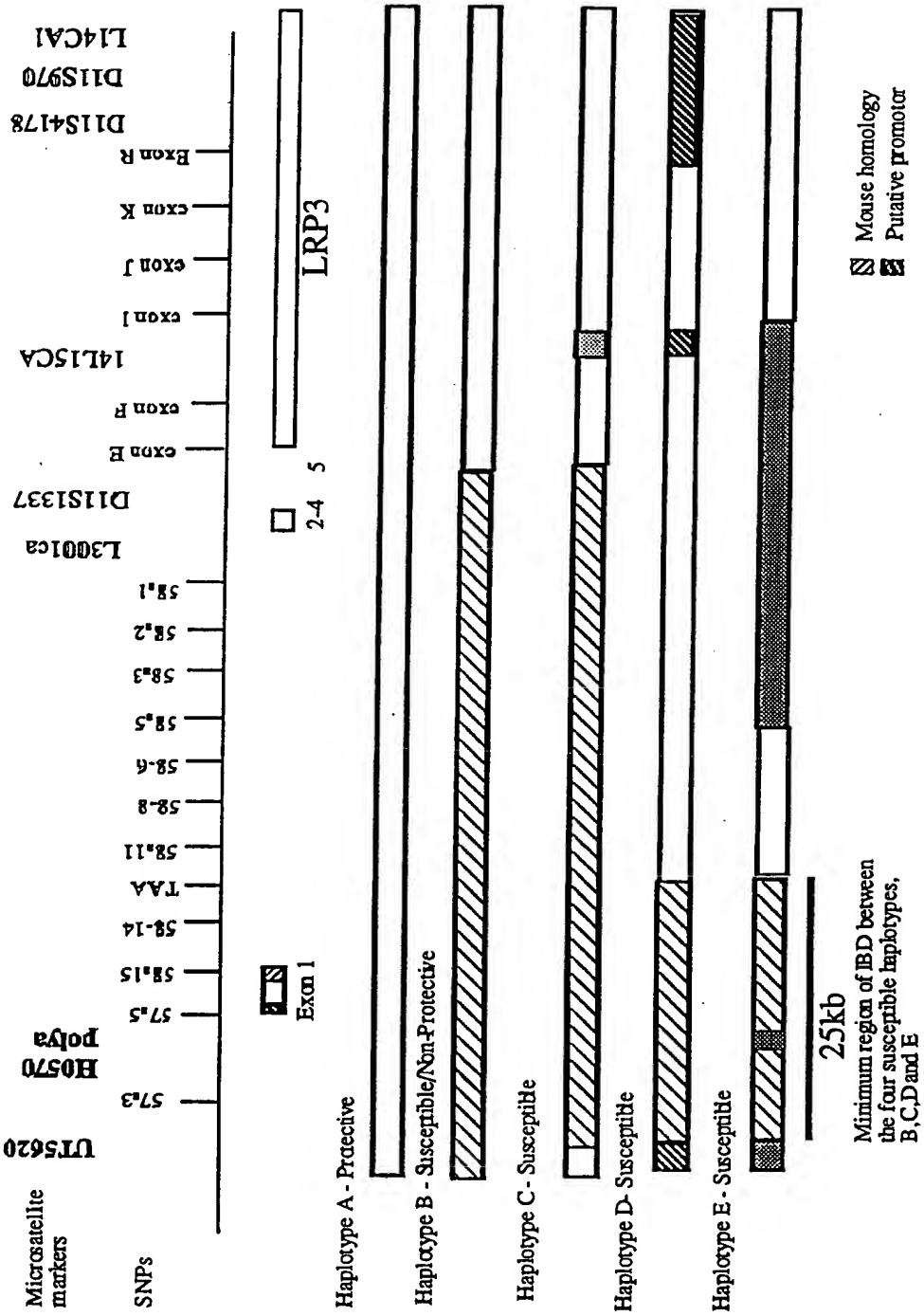

255ca5
D11S987
255ca6
255ca3
D11S1296
EO864ca
100
UT5620
HO570polya
TAA
58.1
L3D01ca
D11S1337
200
R Exon
14Lca5
D11S4178
D11S970
Exon R
L14ca1
300kb
LRP3
18018
Haplotype A - Protective
Haplotype B-Susceptible/Non-Protective
Haplotype C - Susceptible
Susceptible/non-protective haplotype
Protective haplotype
Minimal region of IBD between haplotypes B and C

Figure 19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SNPs
UT5620
H0570
polya
57a.3
57a.5
58a.15
58-14
TAA
58a.11
58-8
58-6
58a.5
58a.3
58a.2
58a.1
L30001ca
D11S1337
exon E
exon F
14L15CA
exon I
exon J
exon K
Exon R
D11S4178
D11S970
L14CA1
LRP3
2-4 5
Exon 1
Haplotype A - Protective
Haplotype B - Susceptible/Non-Protective
Haplotype C - Susceptible
Haplotype D- Susceptible
Haplotype E - Susceptible
25kb
Minimum region of IBD between the four susceptible haplotypes, B,C,D and E
Mouse homology
Putative promotor

Figure 20

INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/GB 98/01102

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/11 C12Q1/68 C07K14/705 C07K16/28
A61K38/17 A61K39/395 A61K48/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HILLIER L. ET AL.: "The WashU-Merck EST project, AC AA203279" EMBL DATABASE, 30 January 1997, XP002076211 Heidelberg see the whole document ---	4,5
X	WO 95 30774 A (BECKMAN INSTRUMENTS INC ;CASKEY CHARLES THOMAS (US)) 16 November 1995 * see SEQ ID NO:17 * ---	11
X	UNIV LEICESTER: "PCR primer WG2G4B, AC Q95283" EMBL DATABASE, 9 February 1996, XP002076212 Heidelberg see the whole document ---	11
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 September 1998	21/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/GB 98/01102

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	RIBOZYME PHARM INC.: "AC T52084" EMBL DATABASE, 24 March 1997, XP002076213 Heidelberg see the whole document ---	11
X	VAN DER ZEE A ET AL: "Genomic cloning of the mouse LDL receptor related protein/alpha 2-macroglobulin receptor gene." GENOMICS, (1994 SEP 1) 23 (1) 256-9. JOURNAL CODE: GEN. ISSN: 0888-7543., XP002076214 * see esp. fig. 3 * ---	13
A	DAVIES J. ET AL.: "A genome-wide search for human type 1 diabetes susceptibility genes" NATURE, vol. 371, 8 September 1994, pages 130-136, XP002076215 cited in the application see the whole document ---	1-38
A	LUO D.: "Confirmation of three susceptibility genes to insulin-dependent diabetes mellitus: IDDM4, IDDM5, and IDDM8" HUMAN MOLECULAR GENETICS, vol. 5, no. 5, 1996, pages 693-698, XP002076216 cited in the application see the whole document ---	1-38
A	TODD J. AND FARRALL M.: "Panning for gold: genome-wide scanning for linkage in type 1 diabetes" HUMAN MOLECULAR GENETICS, vol. 5, 1996, pages 1443-1448, XP002076217 cited in the application see the whole document -----	1-38

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/01102

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 38 is directed to a method of treatment of the human/animal body, the search has been carried out and based the alleged effects of the compound/composition.
effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/GB 98/01102

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9530774 A	16-11-1995	AU 2360195 A	29-11-1995
		DE 69503126 D	30-07-1998
		EP 0758403 A	19-02-1997